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A PHYTOPHTHORA FOOTROT OF RHUBARB[1]

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INTRODUCTION

Rhubarb footrot is a parasitic fungous disease which manifests itself by a wilting of the leaves, followed shortly after by their collapse at the base of the petioles and the rapid death and decay of the whole plant. It is caused by a species of Phytophthora heretofore unreported in connection with this crop. The disease was first brought to the writer's attention in July, 1917, when a report was received in the Office of Cotton, Truck, and Forage Crop Disease Investigations, of the Bureau of Plant Industry, of a disease of rhubarb in a garden in Brookland, D. C., which was killing out the rhubarb plants. Soon afterwards other reports from different points in the District were received. A survey of the environs of Washington showed that the trouble was rather widespread.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Inasmuch as this disease has not yet been reported on rhubarb and is not widely known among the plant pathologists of the country, only a very incomplete account of its distribution can be given at this time. Furthermore, some of the citations given are necessarily based upon inaccurate identifications, as will be seen, and must serve rather as an indication of localities where the disease may be found than as reliable data on distribution. Owing to a change of duties it has become necessary to submit for publication results as they stand without carrying to a satisfactory conclusion some of the doubtful phases of the investigation.

The disease was observed repeatedly by the writer during 1917 and subsequently in various gardens in the District of Columbia and in neighboring Maryland and Virginia. One newly planted field was seen that was almost entirely killed out. Numerous reports of the dying out of rhubarb plants in this same locality, particularly in small gardens, have also been received, and from the descriptions given, either orally or in writing, it would seem that the cause has been the same as in the cases personally studied. Letters and diseased specimens also were received from more distant points. On September 5, 1917, a specimen was received from Harrisburg, Pa., which was similar in appearance to the Phytophthora footrot familiar to the writer, and which was found to contain in affected parts hyphae of Phytophthora. While the fungus

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was not isolated, it was found in mixed culture to belong to this genus. In November of the same year a similar specimen was received from Amelia Court House, Va.

These several occurrences of the trouble in 1917 led to an examination of the card records of diseased rhubarb specimens and of correspondence received by the Office of Cotton, Truck, and Forage Crop Disease Investigations in previous years. Many cards were found upon which the descriptions recorded compared favorably with that of the disease caused by this Phytophthora. On March 24, 1913, a specimen was received from Bowling Green, Ky., which consisted of "decay of underground stems." On January 24, 1914, a specimen of what was probably forced rhubarb was received from Parkersburg, W. Va. It was called a "crown rot" and described as follows:

> Tissue at base of leaves soft and decayed, decay extending into root; bacteria abundant.

Reference to the paragraph in this paper headed "Description of the Disease" will show that abundance of bacteria is characteristic of the later stages of footrot rather than indicative that bacteria are in any sense primary. On July 31, 1914, a specimen from Mount Rainier, Md., was described as a "'crown rot' . . . tissue full of bacteria." On September 2, 1915, specimens from Richmond, Va., were "affected with a basal stem decay." On August 3, 1916, a specimen "in bad condition" was received in the office from a garden in Washington, D. C. In January, 1920, a letter was received from Cobden, Ill., telling of the occurrence of a disease in rhubarb fields that had killed out a large number of plants. The statement was made that replantings made in the spring of 1919 into hills where rhubarb had died the year previously were killed by the disease that same summer. In the summer of 1921 specimens sent from St. Louis, Mo., showed typical symptoms and the presence of a Phycomycete, probably Phytophthora, in the roots. In addition, reports of rhubarb diseases with no particular clue as to their identity were received from time to time from Kentucky, Tennessee, Missouri, and Idaho.

The earliest report of which the author has been able to find any record was contained in a letter dated July 20, 1902, from Prof. William Stuart, then at Purdue University, La Fayette, Ind., in which he described a disease of rhubarb similar to the one under investigation. Specimens accompanied the letter, but the correspondence shows that they were so much decayed that it was impossible to determine definitely the nature of the trouble.[2]

Beach (*2*),[3] who has made the only mention of such a disease to be found in the literature, reports in his paper and by letter that in the summer of 1920 a rhubarb crownrot due to Phytophthora was prevalent in Philadelphia County, Pa., in one case having killed nearly half the stand in an acre field. As will be shown later in this paper, however, his disease is not identical with that investigated by the writer, the causal organisms being distinctly different.

This fact complicates the matter of distribution considerably, inasmuch as the two diseases appear to be very similar if not indistinguishable. The other organism, or one very close to it, has also been received

[2] An extract from the letter follows: "The rhubarb plantation from which the plant sent you was taken is quite badly affected. The gross characters of the disease in affected plants is the wilting of a leaf or two until finally all are affected, when the crown turns dark and decays. The decay of the crown terminates the existence of the plant. The owner of the rhubarb bed said that he replaced all those that had died the year previous, only to find that the newly set plants soon became affected and succumbed to the disease."

[3] Reference is made by number (italic) to "Literature cited," p. 24-26.

from Ithaca, N. Y., where it was isolated from rhubarb in two separate gardens. It is evident, therefore, that the data here given on distribution may have to do with the disease caused by either one of the organisms, except in those cases in which the specific organism is clearly noted. It is hoped that it will be possible, after another season's observations, to distinguish between the diseases under field conditions. Mr. Beach has much additional data in this connection which will probably be published soon in a bulletin of the Pennsylvania Agricultural Experiment Station.[4] His paper is an important and valuable contribution to this and other phases of the rhubarb crownrot problems.

It is not possible to make any estimate of total loss at this time. Since rhubarb is rather extensively grown on a commercial scale and the disease is widespread, it will have to suffice to say that the footrot is of considerable economic importance.

DESCRIPTION OF THE DISEASE

The first outward manifestation of Phytophthora footrot is a sudden wilting of the foliage, usually of one or two leaves only. The base of the petiole, or leaf stalk, is the point affected. Here a distinct lesion is found, slightly sunken, sometimes not colored, and sometimes in sharp contrast with the pink or light green of the normal petiole because of its brown color. A leaf that has reached the wilting stage is shown in Plate 1, A. The lesion has practically girdled the base of the stem. This area advances rapidly, and in less than 24 hours the stem may collapse at this point, allowing the leaf to fall to the ground. Leaf stalks with the disease slightly farther advanced are to be seen in Plate 2. The tissue is killed so quickly that secondary organisms, particularly bacteria, gain entrance almost immediately. These contribute to the rapid decomposition of the fallen leaf, turning it quickly into a putrid mass, especially at the base, as shown in Plate 2. This factor makes it difficult to isolate the causal organism. Fungi as well as bacteria play a leading part in the further destruction of the leaf. A species of Colletotrichum, probably the same one treated by Stevens (21) and considered by him to be *Colletotrichum crumpens* Sacc., is found in great abundance at times on decumbent rhubarb leaves and petioles in early and late stages of decomposition. Plate 3, B, illustrates a leaf petiole recently fallen with Colletotrichum appearing along its entire length. Plate 3, A, shows a leaf entirely covered with this fungus in a fruiting condition.

In weather favorable to the disease the first symptoms are followed in a day or two by the infection and collapse of other leaf stalks in the plant, as shown in Plate 4, A. Finally, all the leaves drop, leaving the plant entirely dead (Pl. 4, B). Meanwhile other plants in the field are similarly affected and many of them are killed, so that the field may show large vacant areas.

A study of many plants in different stages of infection showed that the fungus advances into the root from affected leaf stalks, causing a brown decay. Thence it spreads rapidly through the upper portion of the root, killing the other leaves and leaf buds. Plate 5, A, a section through a recently killed plant, shows the advancing edge of decay due to the causal organism. At the points marked (X) the fungus was found and identified as Phytophthora. Plate 5, B, shows clearly how the fungus,

[4] This bulletin has since appeared: BEACH, W. S. THE CROWN ROT OF RHUBARB CAUSED BY PHYTOPHTHORA CACTORUM. Pa. Agr. Exp. Sta. Bul. 174, 28 p., 25 fig. 1922. Bibliography, p. 27-28.

entering the root from one side, spreads and involves the whole plant. The remaining stalks are doomed to quick collapse, for the fungus has advanced from the other side of the plant, which has already been killed, and has penetrated the upper portion of the roots feeding this side. Within a few hours these stalks would present the appearance shown in Plate 4, B.

This disease should not be confused with one very commonly found in rhubarb fields during the summer months, which consists of a dry necrosis of the base of the petiole and its consequent partition from the plant. The latter condition is pictured in Plate 3, C. It is in no way related to the Phytophthora disease, even though stalks so parted from the plant may appear the same (except for more marked yellowing of the leaves), and may go through identical processes of further decomposition and decay.

Rhubarb shows a remarkable power of withstanding and checking further advance of the disease if conditions are not entirely favorable to the causal organism. A change in the weather may prevent the spread of the fungus from a point of primary infection to the rest of the plant. In artificially inoculated plants in which the fungus was known to have entered the roots, however, the writer has seen the disease continue in its destruction even during a period of dry weather.

INFLUENCE OF WEATHER CONDITIONS ON PROGRESS OF THE DISEASE

Marked variation in the prevalence of this disease from year to year was noted. This appears to be due to differences in weather conditions, especially in the rainfall during the period of greatest danger. In 1917 reports of the disease were numerous, and it was found by personal surveys to be rather general. During the next summer very few reports of rhubarb diseases of any kind were received. Field trips into localities that had been badly affected in 1917 did not bring to light any of the disease in its active condition. During the summer of 1919 the writer had no opportunity to investigate its prevalence, but the appearance of the experimental field the next spring indicated rather heavy loss of plants. In 1920 it appeared worse than ever, as was evidenced by numerous oral and written reports and by personal observations. A field at Arlington Experimental Farm, Va., just across the Potomac River from Washington, D. C., was attacked and plant after plant killed by the disease.

A study of the climatological data for Washington, D. C., and vicinity over the summer months of 1917 to 1920, inclusive (*26*), disclosed some interesting facts with regard to rainfall. The total precipitation for June, July, and August, 1917, was 16.43 inches; for the same period in 1918, when the disease was not to be found at all, it was 7.73 inches, or less than half that of the previous year; for 1919, 13.65 inches; and for 1920, 15.21 inches. It is a prolonged period of continuously wet weather, however, rather than a high total precipitation, that is favorable to the development of the downy mildews in general. In this respect, particularly, the weather records show an interesting correlation with the prevalence of the rhubarb disease. In July, 1917, two prolonged periods of daily rainfall and cloudy weather occurred. During the first, from July 8 to July 11, an average of 0.36 inch fell every day, followed by several cloudy days. Again, on July 22, 1917, 0.59 inch of rain fell, followed by two cloudy days (49 and 46 per cent of possible sunshine), and on the next

day 3.27 inches, followed by two cloudy days. During either period conditions undoubtedly favored the development of a downy mildew. During the summer of 1918 no such period of prolonged wet weather occurred. In 1920, when the next observations on the disease were made, several wet periods occurred, the longest of which was from July 15 to July 23, every day being listed as cloudy. In fact, only one day, the 19th, showed as high as 40 per cent possible sunshine. During these nine days 5.6 inches of rain fell. It was during the latter part of the period that the rhubarb disease appeared at its height.

The extensive occurrence of rhubarb footrot only during such weather conditions agrees with the observations of other workers on other diseases of the same type. Clinton wrote in 1905 (6):

> The prevalence of most parasitic fungi is largely influenced by the character of the weather, particularly in regard to moisture. This is especially true of the downy mildews. An abundance of cloudy or rainy weather at certain periods of the year determines whether or not these troubles will be injurious.

CAUSAL ORGANISM

ISOLATION

When investigations on the disease were started, several organisms had to be isolated and studied before the true cause was discovered. Prominent among these was *Colletotrichum erumpens* Sacc., which has already been mentioned as being almost universally found on decumbent rhubarb stems. This fungus was reported by Stevens (*21*) as the cause of a rhubarb disease in Illinois in 1919. One feature that cast suspicion on it in particular was that it was found fruiting in distinct lesions, such as are shown in Plate 3, B, on freshly fallen rhubarb stems. Furthermore, it appeared to be constantly associated with the footrot; the abundance of infective material on old and dried as well as on freshly fallen leaves would seem to be conducive to such damage as was noted. Single spore isolations were made after considerable difficulty to free them from bacteria, and inoculations were made upon living plants in the greenhouse, under most favorable conditions as regards moisture and temperature. Results were consistently negative. Stems cut and inoculated in a moist chamber became infected, but the symptoms differed essentially from those of the footrot disease. Several bacteria were isolated and used for inoculations without positive results. The finding of a coenocytic fungus in the tissues of fresh material at this time led to a concentration of effort on that and abandonment of further systematic study of the other organisms.

This Phycomycete, after its presence was first noted in newly collected material, was constantly found associated with the typical disease. Repeated attempts were made to isolate it by the tissue fragment method on poured plates of corn meal and other agars. While it grew very readily in most of the media used, it was always accompanied by contaminating bacteria, often even out to the very tips of the growing hyphae. Finally in September, 1917, pure cultures were obtained. On corn meal agar plates the organism, contaminated as it was with bacteria, sometimes made a rather profuse aerial growth. By cutting sheets of sterile agar from a poured plate and laying them one on top of another, little inverted pyramids were built on the under surface of the cover of a Petri dish. These were turned with the cover so as to be directly over the colonies with good aerial growth. In two cases out of several attempts

the fungus grew into contact with these blocks of agar, which were soon after turned again over a clean part of the plate and after several hours' growth had been permitted, were found to be free from all contaminations. These sources were used to inoculate several clean plates, and from them a large number of cultures were made on corn meal, oatmeal, and lima bean agars. Two more strains indentical in character with the first were obtained in this manner, one from a garden and the other from a commercial field, both in the District of Columbia.

PROOF OF PATHOGENICITY

A large number of rhubarb seeds were planted in flower pots in the Washington pathological greenhouses, and about the middle of October, 1917, they were large enough for some preliminary inoculation experiments.

INOCULATION No. 1.—A seedling planted August 31 was inoculated on October 19 by puncturing with a sterile needle a leaf petiole near its base and applying a small bit of a culture from a corn meal agar tube. Five days later, not only the inoculated leaf but all the others on the plant had fallen to the ground. Plate 6, A, shows this plant, together with the control similarly treated except for the application of the fungus, photographed October 26.

INOCULATION No. 2.—On October 19 a similar plant was inoculated by the application of the culture without injury at the base of a leaf stalk. The plant was covered with a bell jar which was removed in 24 hours. This treatment was applied to two plants. On October 24 one of the plants was dead, and the other showed the outer leaves wilted down. The control was perfectly normal.

INOCULATION No. 3.—A plant similar to the others was inoculated as in No. 2, but the bell jar was not applied, moist earth being piled up around the inoculum. At the expiration of five days this plant was dead.

INOCULATION No. 4 was identical in method and results with No 2., the plants being killed and the controls remaining healthy. In inoculation No. 5 the root was injured, and the inoculum applied at this point resulted in its decay and the death of the plant as before. In No. 6 the fungus was applied to the root without injury, and at the end of five days the plant was still healthy. In No. 7 the root was injured as in No. 5, with similar results. In No. 8 the petiole was injured, no bell jar was used, and infection was positive. Controls of No. 5, 6, 7, and 8 remained healthy.

On November 24 an artificially infected seedling inoculated about three days previously and showing typical signs of the disease was removed from the greenhouse and an attempt was made to reisolate the organism. One leaf stalk showed a lesion extending up the stem about ¾ inch and a rot extending into the root from ½ to ¾ inch on one side. In a microscopic mount from the advancing edge of the decay, hyphae of Phytophthora could be seen apparently free from other organisms. Saprophytic fungi, bacteria, and nematodes were abundant at the points of earliest decay. Isolation attempts on rhubarb juice agar and potato agar plus rhubarb juice, made by the tissue fragment method, failed to get the organism into pure culture, though the Phytophthora developed and fruited.

These results were so strongly indicative that the right organism had been found as to justify confining further operations practically to this fungus.

Pathogenicity was completely proved later. Meanwhile a large number of rhubarb roots of standard varieties had been obtained from a seedsman. These were planted, both in a field plot and in flowerpots in the greenhouse, at Arlington Farm. In the greenhouse further inoculations were made on the large plants obtained from roots, and these were almost invariably successful, resulting in the majority of cases in the death of the whole plant and the decay of the root. Inoculations made into both the aerial parts and the roots gave this result. Plantings made in the spring in the commercial grower's field to replace plants that had been killed by the disease the previous season resulted in the decay and death of the new plants. Inoculations into large, vigorous plants in the field at Arlington Farm, made by applying the fungus from pure culture at the base of a stalk where moisture is abundant and growth is rapid, resulted not only in the death of the particular stalk inoculated but in the rapid destruction of the entire plant.

It became necessary to discontinue the investigation of this problem about midsummer, 1918, in order to take up important war emergency work. Except for the maintenance of cultures and occasional observations, it was not until the summer of 1920 that the writer was again able to devote any considerable attention to the problem. Early in July, 1920, the experimental field at Arlington Farm was in vigorous growing condition, and none of the disease was evident. 'Another series of inoculation experiments was started under these conditions. When the inoculations were made the weather was not altogether favorable for infection, and water and shade were applied artificially, as indicated below. A period of wet weather followed immediately after, however, and this resulted not only in the rapid development of the fungus in plants inoculated but also apparently in its spread to other plants. The experiment is reported in detail in Table I.

TABLE I.—*Inoculations of rhubarb plants with Phytophthora parasitica var. rhei.*

Inoculation No.	Date.	Method.[a]	Part of plant.	Location.		Results.	Remarks.
				Row.	Plant.		
[b] 9	July 6	Culture applied without injury	Center, on young leaves and stalks	I	5	July 9, negative	
10	...do	Zoospores	Young leaves, and petioles	I	5	July 9, positive	Reisolation made.
11	...do	Culture applied without injury	General, at bases of leaf stalks	I	6	July 12, positive	Photographed.
12	...do	Culture applied with puncture of scalpel	General, as above	I	9	July 9, positive	Spreading.
13	July 9	Culture applied with injury	General, in crown, after pulling 3 stalks	III	4	July 12, positive	
14	...do	...do	Local, at bases of particular stalks	II	7	...do	Photographed; reisolation made.
15	...do	...do	...do	II	8	...do	
[c] 16	July 12	Zoospores, without injury	Young leaf	IV	1a	July 16, positive	In spots.
17	...do	Culture applied with injury	Base of stalk	IV	1b	...do	Stalk wilted down.
18	...do	...do	...do	IV	1b	...do	Do.
19	...do	Zoospores applied without injury	Leaf, petiole, and base	IV	1c	...do	In local areas, spreading.
20	...do	...do	...do	IV	1c	...do	Do.
Controls.							
9	July 12	Some injured, others not; marked	Other leaves	IV	2	July 16, negative	No infection
10	...do	Bordeaux mixture 4-4-50 applied	On special, young leaves	IV	3	...do	No injury from the Bordeaux.

[a] In general, inoculum was applied by transferring directly from the culture tube with a sterile needle. Zoospore suspensions were poured directly from tubes.

[b] The inoculations were made at 5.30 p. m. on a clear, sunny, and windy day. The plants were sprinkled with tap water from a sprinkling can, and newspapers held in place with weights were spread about the plant to provide shade.

[c] On July 12 the sun was shining and a dry wind blowing at the time the inoculations were made. A large wooden box was placed over the entire series and left there till final observations were made as noted.

Inoculations No. 21 to 28 were made similarly, except that the inoculum was applied in each case to the crown at the points from which stalks were pulled. From three to five points were inoculated in each plant. Immediately after the plants were inoculated every other plant was sprayed at its base with 4–4–50 Bordeaux mixture, the intent being to determine whether plants could be protected from infection by this means. Inoculation and spraying were done on July 12. Five days later observations were made and final notes were taken in the spring of 1921. Eventually three of the four plants inoculated and not sprayed succumbed to the disease. Spraying served to prevent infection, for none of the sprayed plants died.

In connection with the motion-picture film illustrating zoospore emergence, mentioned by Godfrey and Harvey (*11*) another inoculation, No. 29, was made on July 20. Three or four stalks were pulled from a large plant and inoculum was applied from a Petri dish culture at the broken stubs in the crown. This inoculation resulted in the spread of the organism to other parts of the plant and the ultimate death of the plant. Plate 6, B, shows this plant photographed on July 26. Plate 4, B, is from a photograph of a plant that became diseased as the result of inoculation 11 (Table I) photographed July 17.

As indicated in the tabulations after inoculations No. 10 and 14, reisolations were made from affected parts. In the case of the leaf inoculated by application of zoospores (No. 10) reisolation was accomplished by cutting out a portion of the base of a large leaf vein that had become infected, washing it for about a minute in mercuric chlorid 1 to 1,000 and then in three changes of sterile water, and applying in pieces broken apart with sterile forceps to an agar plate. This resulted in a pure culture of the same organism as was used in making the inoculation. An isolation was made from the base of the affected stalk in No. 14 in practically the same way. One of the bits gave a pure culture. These cultures were used for successful inoculations in the greenhouse during the following winter. Thus Koch's rules of proof of pathogenicity were completed and this organism was established definitely as the cause of the disease.

Still further inoculations and reisolations were made in the spring of 1921 on some potted rhubarb plants in the greenhouse. At this time an easy method of isolation to free quickly from contaminating organisms was worked out. On the chance that it may be useful to other workers on Phytophthora, this method is here described. It consists simply of inoculating with the mixed culture a good sound apple, according to the methods described completely in connection with another phase of the problem on page 15 of this paper. The Phytophthora quickly outgrew the contaminating bacteria and in about two days was obtained absolutely pure by transferring from the advancing edge of decay in the interior of the apple. Plate 7, B, shows the fungus in pure culture, growing from such transfers of apple tissue, compared with the contaminated culture A, made by transferring from near the point of inoculation.

DESCRIPTION OF THE FUNGUS

So much has been written from time to time of the characters peculiar to members of the genus Phytophthora and of their behavior in culture that an effort will be made here to avoid useless repetition by reference to previous papers wherever possible. An exception will be made of

points upon which emphasis is deemed desirable, and, of course, of any new points that may be brought out.

Morphologically distinct sporangiophores of this fungus have never been observed by the writer in nature. Sporangia have been observed, but always attached to hyphal branches that could not be distinguished by any morphological characters from other aerial hyphae of the fungus. In pure culture this same condition seems to exist. Attempts were made with the use of Van Tieghem cells to produce sporangiophores, as was done by Rosenbaum with several different species of Phytophthora (*21*). In the aerial hyphae that developed, sporangia could be seen on irregularly branched hyphae. In no case were clusters of sporangia seen, such as are pictured by Rosenbaum. Plate 8, D, E, F, and Plate 9, G, H, are camera-lucida drawings of sporangia as found in pure cultures attached to the hyphae.

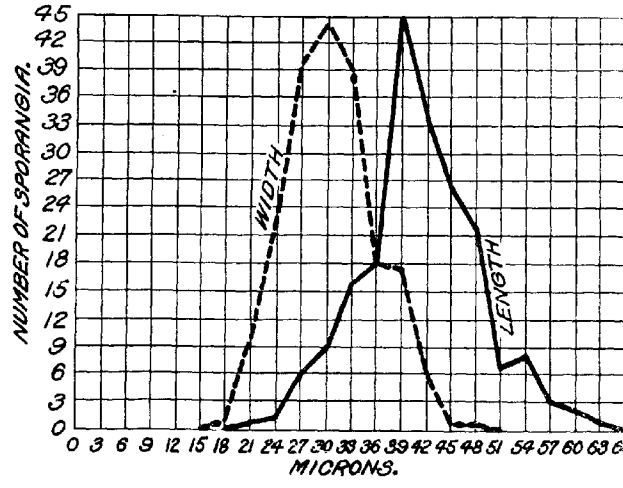

FIG. 1.—Graph showing the variation in length and width of sporangia of *Phytophthora Faberi* var. *rhei*.

Sporangia are variable in size and shape but on an average would be said to be comparatively large, and of the short, ovate type rather than the long, slender kind. Figure 1 shows the results of 200 measurements and shows graphically the sizes of greatest frequency. It will be seen that practically all of them fall within the limits of 33 to 48 μ by 24 to 39 μ, the mode being 39 by 30 μ, the average 41.03 by 30.85 μ. The ratio of length to width varies from 1.2 to 1.5, 1.3 occurring more than any other. Papillae are mostly apical, an occasional lateral one being found. They are prominent, the average of 25 measurements being 2.75 μ in height and 5.9 μ in width. Sporangia are mostly terminal, but frequently intercalary. Intercalary sporangia are sometimes normal in both size and shape, but many functioning sporangia have been observed, very small, spherical, and lacking the apical papillae. Such were considered out of the ordinary and were not included in spore measurements. The wall of the sporangium gives the true cellulose reaction to the chloroiodid of

zinc stain, described by Stevens (*25, p. 300*) and other authors. The igures in Plate 10, E, G, and H, are photomicrographs of typical spo-angia before the escape of zoospores. These figures bring out clearly he apical papillae. Figures C and I in Plate 8 are camera-lucida draw-ngs of typical sporangia.

Chlamydospores are sometimes very abundant in pure culture. They re spherical, thick-walled as compared with sporangia, and in some nedia slightly brownish in color. They, too, may be either terminal on he ends of short branches or intercalary. They are relatively large, neasuring from 27 to 39 μ in diameter, 33 μ being the mode, as indicated by Figure 2, depicting the results of 200 measurements. Plate 10, F, is

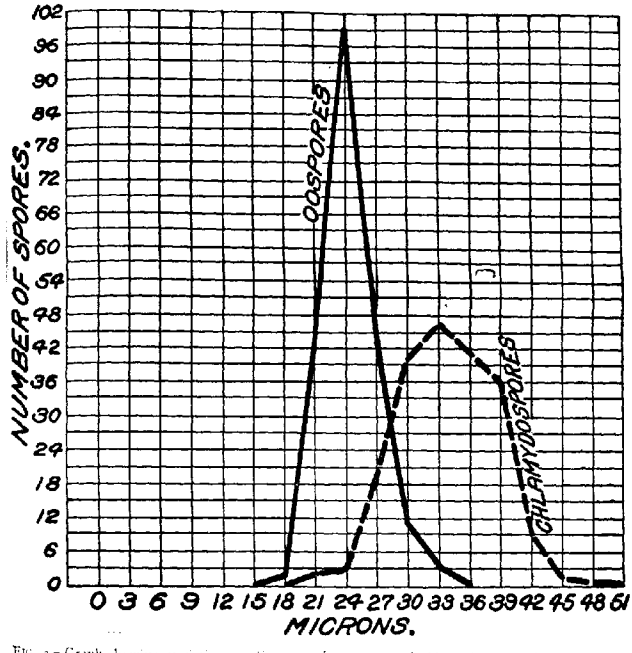

FIG. 2.—Graph showing variation in diameter of oospores and chlamydospores of *Phytophthora Cactorum* Var. *Rhei*.

a small, laterally attached chlamydospore, and Plate 8, A, one that is ntercalary.

Oospores are also found abundantly in pure culture, especially in some of the more nutrient media, such as oatmeal or lima bean agar, though hey have been observed very thickly at times in corn meal agar, in the mall piece of the medium with which the transfer was made from one ube to another. Oospores are spherical, ranging from 21 to 27 μ in dia-neter, the mode being 24 μ (fig. 2) and the mean a fraction less than 25 μ. The type of fertilization of the oogonium is always amphiginal, as de-cribed originally by Pethybridge (*18, p. 536*) and shortly after by Dastur

(*10*). Figures A to D, in Plate 10, are photomicrographs of typical specimens. Two views of the same oospore are shown here, A with the focus on the stalk of the oogonium and B on the oospore wall. Another oospore and a sporangium are also in the field, but neither is in focus. C shows especially well the relation between oogonium and antheridium, arising from separate hyphae. Plate 8 includes camera-lucida outline drawings of oospores, with the oogonium arising from the same (H) and from different (G) hyphae. Oospores are thick-walled, sometimes hyaline, sometimes distinctly brown, depending upon the substratum. The irregular thickening of the outer wall of the oogonium after the oospore is mature, mentioned by Dastur (*10*, *p.* 202), is often seen in oatmeal agar. The antheridia are variable, having been seen in all gradations from half the diameter of the oospore and almost square in shape to small and circular in outline. Antheridia are persistent.

Hyphae are slender, though variable, and full of granular protoplasm in younger cultures. A rapid flowing or streaming of the protoplasm has been observed under higher magnifications in young cultures on plates of clear corn meal agar. This flowing can be seen for only a few seconds after the cover of the plate is removed; the drying out of the cultures appears to stop it. The type of mycelium varies considerably with different culture media. Various features described in different papers have been observed. There are no septa in the younger hyphae, but in older cultures septations are frequent. Plate 10 shows hyphae as seen in microscopic mounts. Irregularities of the most extreme nature, even approaching the tuberculate structures mentioned by Rosenbaum (*21*, *p.* 24) as a characteristic of *Phytophthora syringae* Klebh., have been seen occasionally. Hyphal walls and septations consist of true cellulose.

Zoospore production in Phytophthora has been repeatedly described. The manner of their development and emergence from the sporangium is in this species apparently identical with that in *Phytophthora parasitica* Dastur as described by Dastur (*10*, *p.* 194). One feature that was particularly noticeable with this organism was the rapidity with which zoospore development occurs when a water mount is made from a culture of the right age and condition. When cultures are maintained at room temperature, zoospore production can be observed at any time within a few minutes. Often less than a minute from the time the mount was made sufficed to note the beginning of activity. Sometimes 15 or 20 minutes are required. Normally, activity begins in less than 5 minutes, during which time the changes in the protoplasm can be observed. This feature, together with the abundance of sporangia produced on some of the ordinary media, the long life of the organism in culture, and other characters mentioned in this paper seem, in the writer's opinion, to make it particularly desirable to use for laboratory study in plant pathology courses.

A motion-picture film has been prepared, as noted elsewhere by Godfrey and Harvey (*11*), partly for the purpose of showing to best advantage the details of the development and escape of the zoospores. Through the courtesy of the motion-picture laboratory portions of that film which show different stages in the process are reproduced in Plate 11. The first row shows the protoplasm rounded out into zoospores, with cleavage lines distinctly visible. This stage is more clearly brought out in Plate 10, H, which shows a specimen killed with osmic acid fumes just previous to the escape of the zoospores, and treated with chloroiodid of zinc (*25*, *p.* 300). This stains the sporangium wall a light blue or purple and the

ontents yellow. Next occurs a sudden bursting outward of the papilla, vhich can be seen in strip B. It was distinctly seen that there is no ıursting of a membrane, but rather a stretching of the apparently gelatiıous substance of the papilla itself into a thin plasma membrane, which ırdinarily remains intact, holding the contents together, until a large ıart or rarely all of the protoplasm is out of the sporangium. The memırane then gives way all at once, possibly by becoming dissolved rather han bursting at any one point, and the zoospores free themselves from ıne another and swim away in all directions. This stage is shown in Plate 11, C. By comparing the extremities of the strip, it will be seen hat change is rapid at this stage. The pictures are taken at the rate of :6 per second. At this stage also flagellae may often be seen lashing out ın all sides of the protoplasmic mass at the mouth of the sporangium. With a large sporangium, which may contain as many as 64 zoospores, hose that escape first in a single body usually become free and swim ıway before the last are out. This condition is evident in E. In such ı case it is interesting to watch the amoeboid movements of the final ıarts of the protoplasmic mass. Figures F and G show the final stages ıf this very interesting phenomenon. Plate 8, B, also shows different ıtages of zoospore emergence, as drawn with the aid of the camera lucida.

Zoospores are so active when first they become free that it is impossible ıo catch any but the faintest glimpse of the flagellae. Many observaıions under different conditions, however, revealed bit by bit the various hases of their activity. Perhaps the best view of them in their normal ictivity was obtained in the slightly dull light of late afternoon, with the lirect light from the blue sky and a 2.5-mm. Zeiss water immersion ens. This particular light appeared to provide the correct conditions or displaying the delicate flagellae. These observations were possibly till further favored by a low temperature, which appears to slow up the novements of the organism.

The zoospores are the usual Phytophthora type, kidney-shaped, bicilate, contents granular, and nucleus indistinctly visible in the unstained pecimens. They measure 8 to 9 by 11 to 13 μ while active, gradually ounding up as they lose their activity. Eventually they are globose ınd measure from 8 to 10 μ in diameter. The flagellae are attached lose together in a longitudinal groove on one side. As the swarm spore wims along with its more pointed end forward, the anterior flagellum s the shorter, being approximately twice the length of the spore. t lashes violently back and forth. The posterior one is about ½ times the length of the spore and drags along without much notion. As the zoospore gradually comes to rest, slight enlargements nay be seen on the ends of the flagellae. As these droplets become larger he flagellum shortens by what appears to be a process of deliquescence. 'his has been observed in connection with some of the water molds and ıas evidently been observed in Phytophthora as well, for Sawada picures it in connection with *Phytophthora allii* Sawada (*22, Pl. 1*). The droplets approach the spore as it becomes quiet and round, and it is presumed that they drop off, though this has not been confirmed by the writer. One spore under observation for 15 minutes showed the droptts still not quite in contact with the spore, and it was lost before any urther change took place. Most zoospores after having come to rest o not have these droplets attached. Plate 9, A to C, are camera-lucida rawings made from fixed materials. A slide showing swarm spores in arious stages of activity was held for about 10 seconds over a bottle of

2 per cent osmic acid. This killed the spores instantly. Then very dilute aqueous gentian violet solution was applied, resulting in the immediate staining of the spores. The flagellae too, being at rest, were distinctly visible, and in certain cases their position would seem to indicate their instantaneous cessation of normal active motion. The osmic acid darkened the oil contents of the spores sufficiently to interfere with the view of the structure of the protoplasm; consequently no attempt was made with this material to draw the cell contents. Figure A of Plate 9 shows spores in varying positions, with flagellae. Figure B shows different stages in their deliquescence and disappearance. Here is seen a rounded spore that has come to rest with the droplets still attached. Figure C shows zoospores that are abnormal or that had not sufficient time, before being killed, to round out into normal.

Within an hour or two after motility ceases zoospores often begin to germinate. Plate 9, D, consists of drawings of germinating zoospores in different stages of growth. Germination in water sometimes results in the formation of small conidia, normal in appearance, as shown in F. These may produce zoopores, or may germinate by germ tubes and continue the vegetative growth. At times also small chlamydospores may be formed (Pl. 9, E). Germination on a plate of corn meal agar resulted in the development of normal vegetative hyphae such as occur when the host plant is infected with zoospores. Figures K, L, and M in Plate 10 consist of photomicrographs of germinating zoospores in different stages.

CULTURAL CHARACTERISTICS

The appearance of this Phytophthora, as of others, differs considerably on different media. It was not grown on an extensive list of media for the express purpose of making comparisons, but on those used for special purposes, observations were made as follows:

Corn meal agar (30 gm. corn meal, 20 gm. agar, 1,000 cc. water). At first growing on and beneath the surface, the organism very quickly (usually by the second day) produces aerial hyphae which, in mass, present a loose, fluffy, semitransparent appearance rather than a pure white, cottony appearance, such as occurs with many fungi producing colorless hyphae. Mature sporangia may be found on the fourth day, though they are not present in greatest abundance until the culture is from 10 days to 2 weeks old. Chlamydospores and oospores may often be found attached to the glass above the surface of the medium, especially if a thin layer of the agar were left there while the tube was being slanted.

Oatmeal agar (100 gm. ground Quaker oats, 20 gm. agar, 1,000 cc. water). The growth is similar to that on corn meal agar, except that the aerial mycelia become more dense. This medium is very good for the development of oospores, which occur profusely in the surface layer after it dries out a little.

Lima bean agar (100 gm. lima beans in 2 per cent agar). Growth is similar to that on corn meal agar, except that the surface and subsurface development is more pronounced and of longer duration before the aerial hyphae develop, and the latter growth is not nearly so dense. This medium is a very good indicator as to the purity of a culture. Bacterial contaminations become evident very soon, if they are present. It is also good for the production of oospores, which are sometimes found in great abundance embedded in the surface layers.

Various sterilized vegetable plugs were used. Dastur has noted (*10, p. 217*) that *Phytophthora parasitica* failed to develop on sterilized carrot and sterilized sweet potato. The writer's strain grew very readily on these media, producing a tough superficial mat of hyphae at first, and after a few days a dense white cottony growth. The growth was similar on string bean plugs and on sterilized potato. On none of these vegetable plugs were sporangia produced to any great extent.

The organism is very tolerant to acid, at least to that found in the rhubarb stem, as might be expected from its habits in nature. It grew in pure culture, though somewhat slowly, in rhubarb decoction that titrated roughly to phenolphthalein 0.07 N (+70 Fuller's scale). Haas (*12*) gives the following figures for the acidity of different parts of the rhubarb leaf stalk; basal part, actual acidity or concentration of free hydrogen ions, determined electrometrically, 0.0007 N; intermediate part, actual acidity 0.0005 N, total acidity 0.1578 N; green part below leaf blade, actual acidity 0.00022 N, total acidity 0.1681 N. As Haas states, there is here shown a great difference between the total and actual acidities, which difference is probably advantageous to the plant's metabolism in that it helps to maintain fairly constantly that actual acidity which is most favorable. Even the actual acidity is unusually high, however, as is shown by the same author in another paper (*13*), in which he compares it with soybean, the former having a P_H of 3.36 (acidity 0.00044 N) and the latter of 5.85 (acidity 0.0000014 N). The acidity, however, does not prevent the active invasion of the Phytophthora into all parts of the stalk, as has been shown repeatedly by observations in the field and inoculations into rhubarb stalks in the laboratory. Neither is high acidity requisite to the rapid growth of the organism, as is shown by its ability to grow on a wide variety of artificial culture media and on many common fruits and vegetable roots.

In the winter of 1917 inoculations were made into the starchy food-storing roots and tubers of various plants. This was repeated in 1920–21 with a wider range of hosts, including certain fruits. The roots and fruits were first thoroughly surface sterilized by immersing for 10 to 15 minutes in 1 to 240 formaldehyde, then wrapped in a dry towel and allowed to stand overnight. The inoculations were made by cutting a deep slit with a sterile scalpel and inserting the inoculum from a vigorous culture about 8 days old. The specimens were then placed in moist chambers and allowed to stand 2 to 4 days.

Stayman Winesap and Grimes Golden apples were both rapidly attacked, the fungus growing in toward the center as rapidly as it spread beneath the skin. The decay of the fruit was light brown or tan in color and of a soft and mealy consistency. Typical hypae could be found at any point in the advancing edge of the decayed area. Reisolations were easily secured free from contaminations, from the advancing edge beneath the skin, or from the interior of the fruit. Seeds that had been reached by the fungus in one of the fruits when planted in a plate of corn meal agar gave a pure culture of it. Parsnips (*Pastinaca sativa* L.) were readily attacked with a soft, wet, but not watery decay and a characteristic pinkish or light purplish discoloration of the surface. Carrots (*Daucus carota* L.) also were rapidly decayed, with a clear watery condition developing darker in color than the normal. The decay spread to the growing leaves and quickly involved them. Turnips (*Brassica rapa* L.) decayed more slowly and did not lose their hard texture, but a distinct blackening of the interior occurred. Sweet potatoes (*Ipomoea*

batatas Poir) were readily attacked, with a firm dryrot, and the production of a distinct rose-geranium odor that is always evident in soft-rot due to *Rhizopus nigricans* Ehr. (*14, p. 341*). White potato (*Solanum tuberosum* L.) decayed rapidly with exactly the same symptoms as those described by Pethybridge for *Phytophthora erythroseptica* Pethyb. (*18*). The pink color, though not evident when the tuber was first cut, developed in the course of half an hour's exposure to the air. This was especially striking when first seen, because of its unexpectedness. It was followed by a change to dark brown as with the other species. Tomato fruits (*Lycopersicon esculentum* Mill), green and ripe, were rapidly attacked, with the spread of the fungus through the interior with brown discolorations. No aerial mycelium developed. Dasheen (*Colocasia antiquorum* Schott) corms were not attacked by the fungus, though the same moisture and temperature conditions prevailed as with all the others. Onion (*Allium cepa* L.) was not decayed, except slightly in the particular layer in which the inoculum was placed.

The whole series was in duplicate, with a third specimen punctured, but not inoculated, as a control. All controls remained intact. Later the entire series was repeated with identical results. Reisolations of the organism into pure culture were made from all except the onion and the dasheen. Plate 12 consists of photographs of the different specimens, showing the nature of the decay produced.

Temperature has a marked influence on the development of the organism. Corn meal agar plates were placed in a series of Altmann controlled temperature incubators ranging from about 4° to 40° C., with intervals of from 2° to 3°. The cardinal temperatures, as indicated by extent of vegetative growth, were approximately as follows: minimum, 13°; optimum, 30°; maximum, 36°. It will be seen that this organism is like the tropical forms in having very high temperature requirements. This explains the facts noted elsewhere in regard to season of active parasitism.

INOCULATIONS INTO OTHER PLANTS

No very wide range of growing plants was inoculated, due partly to lack of time and partly to the unsatisfactory results of previous investigators. In this connection Wilson (*30, p. 77*) says:

> A comparison of the results published by the various authors tends to throw decided doubt upon the value of this method [cross inoculations] of delimiting species in this genus, as practically any species of Spermatophyta, which is in nature subject to the attacks of any Phytophthora, is likely under laboratory conditions to be more or less severely attacked by almost any other species. Indeed, some of the hosts recorded for various species of the genus are not known to harbor these fungi in nature. It would appear, then, that the parasitism of Phytopthora is of such a low order that it will not admit of their being differentiated into races as are certain of the Uredineae for example.

To consider one case: *Phytophthora cactorum* (Lebert and Cohn) Schrot, originally isolated from a species of cactus, was later found in Japan and America (*20*) on ginseng and still later in Europe (*31*) and America (*1, 28*) on apple and pear. Such inoculations may be, however, of direct value in some cases, and a few were made with the rhubarb Phytophthora on hosts of closely related species to determine whether the signs, if any, produced on the plant would be the same as those caused by the fungus originally obtained from it.

Castor bean (*Ricinus communis* L.) inflorescences were cut from plants and inoculated in the laboratory, as was done by Dastur with *Phytophthora parasitica*. Male and female buds were attacked and discolored.

but no fruiting of the fungus occurred. Further inoculations were made with zoospores and with mycelium from a culture, on the young growing inflorescences and leaves of castor beans, numbering six in all. Definite signs of infection resulted, which were, however, confined to small, irregular spots and did not spread. No fruiting of the fungus occurred, even though high humidity and a favorable temperature were maintained. Six inoculations each similarly made on young tomato plants (*Lycopersicon esculentum*) and on young dasheen leaves (*Colocasia antiquorum*) failed to produce infection.

HISTOLOGICAL STUDIES

The time available for the study of the problem was too limited to permit of going deeply into the matter of penetration and infection of the host. One mount was made after the manner described by Vaughan (*27*) of a portion of the epidermis of a rhubarb stalk, at a point recently inoculated with zoospores. This showed hyphae arising from germinating zoospores, growing along the surface, and in one case, at least, penetrating a stoma. Whether this is the regular method of penetration is not completely worked out. After entering the host, however, the fungus is principally intercellular. Figure 3 consists of semidiagrammatic camera-lucida drawings of free-hand cross sections of a rhubarb leaf stalk showing intercellular hyphae and a haustorium. Cell wall penetration takes place occasionally by means of a fine constriction, enlarging after penetrating, to normal size. Figures I and J of Plate 10 show such penetration, as well as the intercellular hyphae. The sections from which these slides were made were cut from a block taken from the base of the stalk shown in Plate 1, B. In the only cases of such penetration actually observed the fungus did not continue to grow through the cell, but appeared to develop into haustoria, both the short, button-shaped and the finger-like forms having been seen, such as those pictured by Dastur for *Phytophthora parasitica* (*10, Pl. III*). The fixed material was killed in Fleming's weak killing fluid, sectioned 6 μ thick, and stained with Pianeze III B stain (*27*). Another stain found by the author to differentiate quickly the fungus within the host was a new

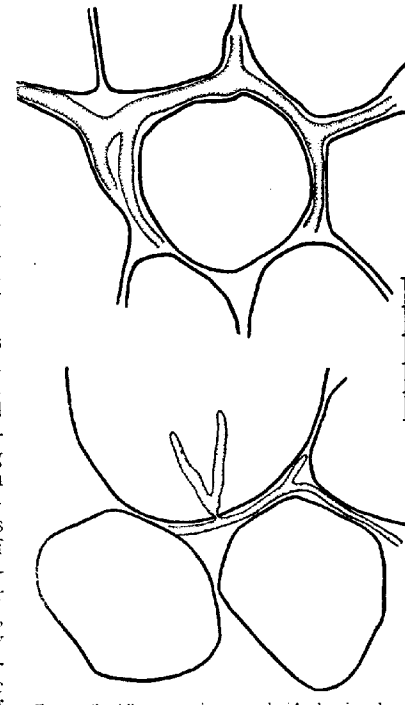

FIG. 3.—Semidiagrammatic camera lucida drawing showing intercellular mycelium and penetration of a cell wall. One space in the scale represents 10 μ.

combination, a modification of a stain used by E. G. Arzberger, of the Office of Agricultural Technology, Bureau of Plant Industry. This consisted of applying with a dropper strong acid fuchsin in 95 per cent alcohol followed by dipping in absolute alcohol till the excess is washed away. Then a saturated solution of Lichtgrün in clove oil is applied. This acts as both a clearing agent and a stain. It removes the red from the host tissue, except lignified tissue such as is found in the fibrovascular rings, replacing the red by light green. The gradual differentiation here can well be observed under the microscope. The clove oil is removed with xylol, and balsam is then applied as usual for making the mount permanent. The duration of the stain is not known to the writer, but mounts several months old are still good.

TAXONOMY OF THE FUNGUS

There are so many characters common to all the described species of Phytophthora that it is necessary carefully to consider each of them before undertaking to determine the proper taxonomic position of a new strain. In this case all but one were eliminated by some definite morphological differences. It was thought well, however, to take into consideration to some extent as well the reactions to different culture media and to different hosts. In the writer's opinion the effect on the starchy roots, tubers, and fruits of certain plants may prove, after more extensive trial, to be very helpful in delimiting species.

No effort will be made here to point out a multitude of differences in any particular case between the rhubarb Phytophthora and the one with which it is compared. It is considered necessary only to give the briefest possible consideration to each case, pointing out one or two clear-cut differences that will suffice to distinguish them.

Phytophthora jagi (Hartig) Hartig, *P. cactorum* (Lebert and Cohn) Schrot., *P. syringae* (Klebh.) Klebh., and *P. nicotianae* Van Breda de Haan, as listed by G. W. Wilson (*30*), can all be set aside on the start as having the paragynous rather than the amphigynous (*17*, *p. 125*) antheridia. In this class also would fall *P. jatrophae* Jensen and *P. jaberi* Maub. (*P. theobromae* Colem.) (*7*) at least tentatively, for the sexual phases of this fungus do not appear to be perfectly understood. Other morphological characters, however, clearly differentiate the species from ours. Here also should be included *P. omnivora* De Bary, for several of the fungi that were included under this name are in this respect like the others mentioned, although Wilson (*30*, *p. 80*) states that "as *P. omnivora* De Bary is here recognized as an aggregate of undetermined affinity, it need be considered no further." Here, too, would fall Beach's rhubarb Phytophthora (*2*), a culture of which was kindly furnished the writer. In the culture, oospores were found with the antheridium distinctly attached at the side of the oogonial pedicel, as with *P. cactorum* (Cohn and Lebert) Schrot. rather than surrounding it. Other cultural characters which will undoubtedly be brought out later by its discoverer distinguish his organism unmistakably from that of the present writer.

Phytophthora palmivora (Butl.) Butl. (*3*, *4*) differs morphologically and physiologically from the writer's species.

There is left, then, the list given by Pethybridge and Lafferty (*10*, *p. 497*) in their paper on *Phytophthora cryptogea* Pethyb. and Laf., all having the amphigynous antheridia, as does the species under consideration, which adds one more to the growing list of species with this character.

Phytophthora infestans (Mont.) De Bary is clearly distinct from the writer's species. Besides the sharp morphological differences, including the enlargements in the conidiophores so typical of that species, the smaller conidia 27 to 30μ by 15 to 20μ, and the larger oospores, 34 to 50μ by 24 to 35μ, there are physiological differences that clearly distinguish them. *P. infestans* produces oospores to only a slight extent, while in rhubarb Phytophthora they are abundant. The optimum temperature for zoospore production with *P. infestans* is from 12° to 13° C., according to Melhus (*16*), while with the writer's they form very quickly at room temperature, the lower temperatures inhibiting their development.

Phytophthora phaseoli Thaxt. also is clearly different, judging by Clinton's account of that organism (*6*). With it, too, there are nodal swellings in the conidiophores, these being entirely absent in the writer's form. The sporangia appear to average somewhat smaller (17 to 35μ by 28 to 42μ) and the oospores considerably larger (26 to 28μ). The antheridia are only temporary with phaseoli, whereas they are persistent with the writer's species.

No very complete account is given anywhere of *Phytophthora thalictri* Wilson and Davis; but from the description given by Wilson (*29, p. 392*) and Clinton's (*7, p. 894*) subsequent reference to it, it seems safe to consider it distinct. In the first place its conidiophores are distinct, developing in fascicles from the stomata; the sporangia are very much smaller than on the writer's species (20 to 27μ by 13 to 17μ), and the oospores, unknown at first and then later described by Clinton, are somewhat smaller (18.5 to 25μ). The fact that it appears to be a leaf-attacking form primarily rather than a soil-inhabiting and decay-producing fungus, indicates in itself that they are different.

Phytophthora arecae (Colem.), Pethyb., found on the Areca palm in India (*8*), has oospores that are much larger (23 to 38μ), the sporangia, too, running considerably larger in both dimensions than those of the writer's species (20 to 45μ by 30 to 71μ).

The rhubarb Phytophthora is similar to *Phytophthora erythroseptica*, ethyb., in some respects. Its effect when inoculated through a wound nto potato tubers is almost identical with that organism. But the onidia are distinctly different, the rhubarb species having distinct apillae, the other not (*18*). The oospores of this form are distinctly maller than those of the other.

The morphological differences are not marked between the rhubarb ungus and *Phytophthora parasitica* Dastur (*10*). *P. parasitica*, however, roduces distinct spots on affected leaves of the castor bean, the under de of which bears conidiophores issuing from the stomata. Inoculations ade with the rhubarb Phytophthora resulted in the appearance of ots different from those produced by *P. parasitica* and did not result the production of sporangia. The growth of the two organisms on the arious media was similar, as indicated in general by Dastur's account *10 p. 211–217*) except that his organism did not give the slightest growth n sterilized slabs of carrot and sweet potato, whereas the writer's pro-uced copious growth on both. This proved later not to be a legitimate omparison, however, for a strain of *P. parasitica* obtained from Dr. C. D. herbakoff, of the Tennessee Experiment Station, grew practically as ell on these media in the form of steamed plugs as did the rhubarb pecies. *P. parasitica*, as reported by Dastur, would not affect tomato ruits, even when they were wounded, while the writer's spread very apidly into the interior of such wounded fruits. Oospores failed to

develop on corn meal agar (*10 p. 225*) while with the writer's they were often seen to form in that medium. Finally, the oospores of *P. parasitica* measure from 15 to 20 μ in diameter, on an average 18.6 μ, which is very much smaller than the writer's, in which the average is about 25 μ.

It seems advisable here to call attention to the fact that the measurements given by Rosenbaum (*21*) for *Phytophthora parasitica* differ essentially from the original description by Dastur. By some mistake he must have received a culture supposedly *P. parasitica* but, judging by his figures and description, certainly different from that organism when compared with the original. A personal examination of both Rosenbaum's culture and a true culture of *P. parasitica* obtained from Sherbakoff further establishes this difference and leads to the surmise that the culture in question is *P. colocasiae* Racib. instead of *P. parasitica*. No attempt was made to verify this supposition.

Phytophthora colocasiae as described by Butler and Kulkarni (*5*), can be distinguished at once morphologically from the rhubarb species. Its sporangia measure 38 to 60 μ by 18 to 26 μ, that is, distinctly elongate, as compared with those of the rhubarb species, which are seldom if ever more than half again as long as broad. Its zoospores are very large (15 to 18 μ by 9 to 12 μ when in motion and 10 to 13 μ when at rest). There are only about 20 to the sporangium. The writer's measure 8 to 9 μ by 11 to 13 μ and 9 to 10 μ in diameter at rest, and as many as 64 may occur in the sporangium. With *P. colocasiae* the sporangia are easily detached from their conidiophores, and a portion of the pedicel remains attached. In the rhubarb fungus they are very seldom seen detached, and never have been observed free, with a distinct pedicel. The oospores are about the same in size and similar in manner of development.

Phytophthora cryptogea, Pethy bridge and Lafferty, (*19*) has oospores of about the same size, averaging 25 μ, and likewise the sporangia are practically the same size, but they lack the apical papillae or at least the papillae are indistinct, which constitutes a distinct difference from the rhubarb form. In addition, a continuation of the growth of the fungus through an empty sporangium, with the formation of a new one either inside the walls of the old or just beyond, often occurs with *P. cryptogea*, whereas it has never been observed for the rhubarb species. It is like the rhubarb species in being actively parasitic when inoculated into apples and turnips but differs in not finding carrots and parsnips equally congenial. One strong point of difference is that it reluctantly produces spores of any kind. The rhubarb species produces all forms very readily.

Phytophthora allii Saw. (*22*), described in 1919 on *Allium fistulosum* L. in Formosa, has distinctly larger sporangia, averaging 49.4 by 36.5 μ, which fell off with persistent pedicel, in this respect, too, differing from the rhubarb species.

Phytophthora meadii McRae (*15*), the most recently described species of Phytophthora, has sporangia distinctly different from the writer's in that they are very much more elongate, measuring 33 to 67 by 14 to 28 μ; moreover, the scarcity of chlamydospores is emphasized, while with the writer's they are very abundant.

We now come to *Phytophthora terrestria* Sherb. (*23*) and *P. melongenae* K. Sawada (*22*). In a personal letter from Dr. Sherbakoff dated February 12, 1921, he stated that it was his desire that his organism be referred to hereafter as *P. terrestris* (the former ending being grammatically incorrect). Pethybridge and Lafferty (*19, p. 497*) have stated as their opinion

that these two species (*P. terrestris* Sherb. and *P. melongenae* K. Sawada) are synonymous but that no definite conclusion could be reached without more complete descriptions or a comparison of cultures. Likewise Butler (*4, p. 82*) has stated that *P. terrestris* is identical with *P. parasitica*. Be that as it may, the sporangia of *P. melongenae*, as noted from Tanaka's translation (*22*), average 42.4 by 33.9 μ, or somewhat larger, and the oospores are from 17 to 21 μ in diameter, or distinctly smaller than the writer's. Other differences determined from a study of the original paper (*22*) with the aid of Dr. Tanaka are the apparent lack of haustoria in *P. melongenae*, and differences in the effect on other hosts, particularly tomato and potato. The oospores of *P. terrestris* are likewise smaller, being 18 to 21 μ in diameter. The conidia and chlamydospores average practically the same. A comparison of cultures affords further evidence that the two differ. *P. terrestris* invariably produces on corn meal agar plates the peculiar "tufted" growth pictured and described by Sherbakoff (*23, p. 125–126*). The rhubarb form on this medium starts almost at once to produce an aerial growth. The contrast between the two is shown in Plate 6, C. Another difference is in their effect on tomato fruits. *P. terrestris* produces a profuse aerial growth on inoculated green fruits held in the moist chamber, as pictured by Sherbakoff (*23, p. 120*). This was verified with the same organism by the writer. Those inoculated with the rhubarb fungus produced no aerial growth except very slightly at the wound itself, its spread being entirely beneath the surface. These fruits were held as parallel inoculations in the same moist chamber with fruits inoculated with *P. terrestris*.

The conclusions to be derived from this lengthy comparison with other described species of Phytophthora are that the strain from rhubarb is distinct from all except what might be called the *Phytophthora parasitica* group. It seems sufficiently like *P. parasitica* Dastur, morphologically, to be included for the time being at least, as a variety of that species. If this is done, then, as Pethybridge and Lafferty (*19*) have suggested, *P. melongenae* Saw. and *P. terrestris* Sherb. should be included in the same category.

A detailed description of the rhubarb organism follows:

Phytophthora parasitica var. rhei, n. var.

Mycelium at first continuous, later sparingly septate; 5 to 15μ in diameter, mostly intercellular, producing haustoria which may be small and subspherical, or finger-like; sporangiophores not distinguishable from the mycelium; sporangia terminal sometimes intercalary, normally ovate and papillate, sometimes spherical and lacking papillae, 21 to 42μ by 27 to 54μ, mostly 24 to 35μ by 30 to 48μ, germinating by zoospores, rarely by germ tube; zoospores biciliate, 8 to 9μ by 11 to 13μ, becoming globoid, 8 to 10μ in diameter; chlamydospores globose, 27 to 42μ; walls thick; oogonia in abundance in old cultures, 24 to 33μ in diameter, pale to brownish in color, penetrating through antheridium which remains attached at base; antheridia variable; oospores, globose, thick-walled, 21 to 30μ, mostly 24μ in diameter; growing profusely on most vegetable culture media.

Parasitic in base of leaf petioles and roots of rhubarb (*Rheum rhaponticum* L.) in Maryland, District of Columbia, and Virginia, United States of America; type locality, District of Columbia; type culture in Bureau of Plant Industry, Washington, D. C.

CONTROL MEASURES

Spraying experiments with Bordeaux mixture 4-4-50 and with Bordeaux dust were started on a small scale at Arlington Farm, Va., in the summer of 1920 during the height of the season favorable for infection. Conditions in no wise favored an extensive spraying experiment at this time, however. In the first place, there was no assurance early enough

in the season of the presence of general infection. When sprays were finally applied, infection was in many cases already present in the plants. Then experimental work in a distant locality compelled the writer to be absent from headquarters for several months, so that when he did return it was too late to get complete data on the work done. A present consideration of spraying as a control measure for this disease, therefore, is more or less theoretical. The erratic occurrence of the disease, because of its absolute dependence upon weather conditions, appears to be sufficient justification for publishing what is available on the subject now, rather than waiting for the uncertain developments of another season.

An examination was made in the spring of 1921 of the field in which the experimental spraying was done. All plants that were alive were well along in their spring growth. It was very evident that a large proportion of the plants had been killed by the disease during the previous season. Of a total of 87 plants alive at the beginning of activities in 1920, 7 had been killed by artificial inoculation. Twenty-five of the remainder had been sprayed, and of these only 3 plants, or 13.6 per cent, had died. Of the 55 unsprayed plants, 25, or 45.5 per cent, were missing. It is certain that many, if not all, of these had succumbed to the disease. This would indicate a beneficial effect from the spraying, in spite of the fact that infection was present in some of the plants when the spraying was done. It gives some ground, therefore, for tentative conclusions in regard to the value of spraying.

Basing conclusions on general information as well as on this experiment, it would seem to be safe to recommend Bordeaux mixture as a preventive against infection during the comparatively short period in the summer when infection may take place. The spray should be applied with some force directly into the crown of the plant, covering the bases of the leaf stalks, and leaving a surplus to soak into the crown and the ground about it Its first effect is, of course, lethal to any of the fungus with which it comes into immediate contact, at least to any not in thick-walled resting condition. There still remains to be determined the effect of copper in different concentrations on such thick-walled spores. The effect of the spray as a fungicide may last over a considerable period. The copper comes into solution gradually; consequently drying merely holds it in the very place where it is needed—that is, in the crown of the plant—to become available when it is needed during the next wet period. The very rain which would normally start the fungus into renewed growth, with the production of zoospores, would also still further distribute and make effective the fungicide. The distribution of the disease from one plant to others in its vicinity, which undoubtedly occurs extensively in nature, would also be prevented by an application of Bordeaux. The spattering of the water which normally distributes the zoospores of the fungus would carry the fungicide with it as well. In the experiments referred to the fungicide applied July 12 was persistent a month later, despite heavy rains that occurred in the interim. No analysis was made, but the abundance of blue stain present indicated that copper was present in sufficient abundance to be effective. The staining of the leaf stalks would not, under practical conditions, be an objection to the spray, since the main harvesting season is over long before the danger season begins. No injurious effects whatever have been observed on any of the sprayed plants. Caution should be observed not to soak the ground too freely or too frequently, for an excess of copper would, of course, injure the ground permanently for any crop.

In a large commercial field there would be no need for spraying the entire plantation unless the widespread occurrence of the disease indicated this need. Application could be confined to those plants in the immediate vicinity of spots where the disease definitely occurred the preceding season. Consequently, a small machine, such as a knapsack sprayer or a small pressure sprayer (which was used by the writer), is satisfactory under ordinary circumstances.

In addition to the spraying, to prevent infection of plants near those definitely attacked by the fungus, the observance of sanitary precautions is exceedingly important. Diseased plants should be dug up and destroyed, with all the dead parts found lying on the ground. Every vestige of the large fleshy roots showing the presence of decay should be removed. Then a heavy application of formaldehyde of the usual strength for soil sterilization, 1 part commercial formalin to 100 parts of water, should be made to the hole where the plant stood. To replant rhubarb without this sterilization would be a waste of time, for the new plant could not be expected to live through the season. Repeated observations have shown this to be the case (p. 2, text and footnote). In a field in which control measures have not been taken, cultivation may spread the disease along the row. In fact, this appears to be one of the means by which spread occurs. Long vacant spaces in the rows in which the disease has occurred indicate this.

This disease is undoubtedly spread widely by means of the root divisions in the ordinary commercial propagation and distribution of rhubarb. To prevent this, one should be sure that his roots have come from a field in which the disease does not occur, or at least from a clean portion of the field. If this can be relied upon, no further measures will be necessary. Where there is a strong chance that roots have come from diseased fields, examination and occasional cutting with a sharp knife will disclose the presence of actual decay in the roots if it exists. Such roots should be discarded. A thorough wetting of suspected roots with formaldehyde 1 to 100, followed by covering them with burlap bags or canvas for several hours, is a still further measure against starting the disease in a new field. A careful experiment by the writer showed that this is a very effective method of killing most of the surface fungi on the roots and that it does not injure the roots for propagating. Rhubarb when dormant is very hardy and will endure rather extreme conditions of cold, drying, chemical treatment, or other unfavorable conditions.

SUMMARY

(1) A disease of rhubarb, caused by *Phytophthora parasitica* var. *rhei*, is reported for the first time. The causal organism is distinctly different from one mentioned by Beach on the same host.

(2) Its distribution has not been determined. It has definitely been found in Maryland, District of Columbia, and Virginia. It is probably much more widely spread.

(3) The disease is primarily a footrot and rootrot of the rhubarb plant, resulting in its rapid and complete destruction.

(4) Warm, wet weather over a considerable period appears to be requisite to the development of the disease.

(5) The organism is readily isolated and grown in pure culture, though special methods of isolating are sometimes required.

(6) Inoculation experiments are described, in which the plants were almost invariably killed by the fungus. Reisolations were made.

(7) The morphology and physiology of the fungus are discussed in detail. All forms of spores found in Phytophthora are readily produced in pure culture. The development and escape of zoospores from sporangia take place very quickly under laboratory conditions, making the fungus a desirable one for ready observation of these phenomena.

(8) The fungus is primarily a decay-producing rather than a leaf-spot-producing organism. It is capable of attacking the roots of many vegetables other than rhubarb but has not been found on any other host under natural conditions.

(9) The mycelium is intercellular though haustoria have been seen within the cells.

(10) The organism is compared in detail with all previously described species of Phytophthora and is seen to be similar morphologically and physiologically to *Phytophthora parasitica Dastur*. It is therefore described as a variety of that species.

(11) Limited control experiments and general observations point out spraying with Bordeaux mixture as a practicable control measure. This should be combined with careful sanitary measures.

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PLATE 1

Rhubarb leaf stalks showing early stages of infection with *Phytophthora parasitica* var. *rhei*.

A.—Note wilting of the leaf and lesion at base of petiole.

B.—An earlier stage of petiole infection than that shown in A.


A
B

A Phytophthora Footrot of Rhubarb

PLATE 2

PLATE 2

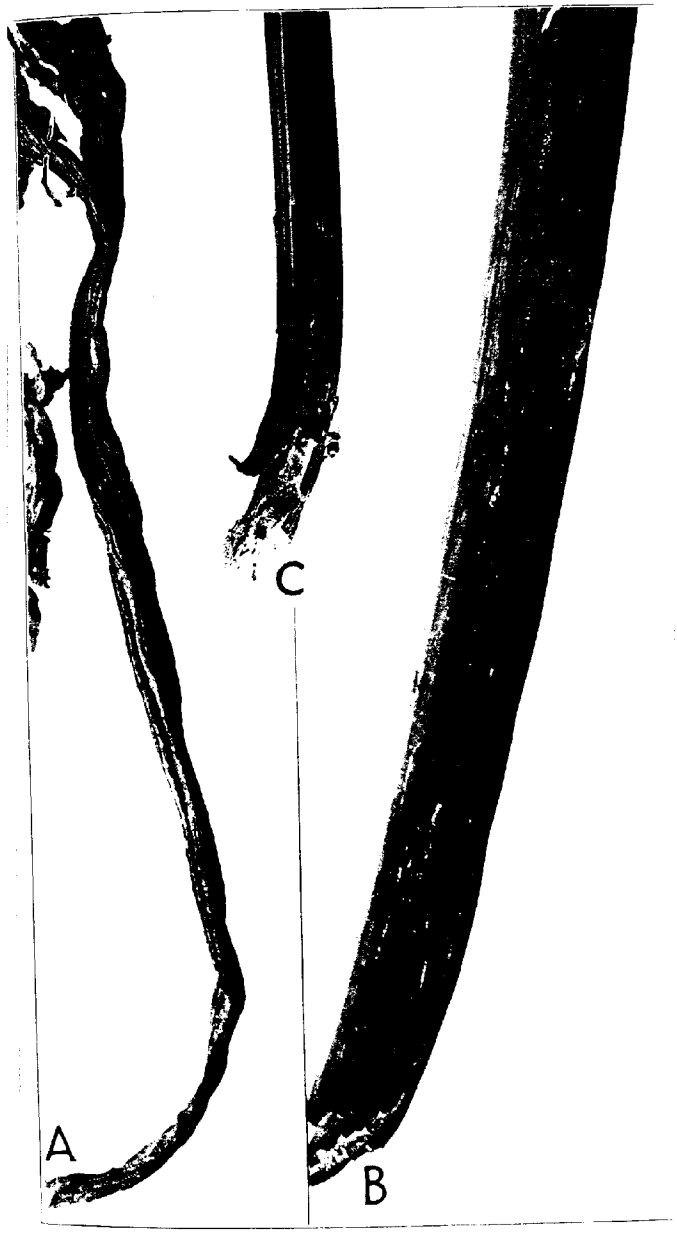
Phytophthora footrot of rhubarb, advanced stage.
The causal organism is present, but contaminated with other fungi and with bacteria.

PLATE 3

A.—A decumbent rhubarb leaf stalk completely covered with *Colletotrichum erumpens*.

B.—Recently fallen leaf stalk, with the same organism just making its appearance in distinct lesions.

C.—Another rhubarb disease marked by yellowing of the leaves and a dry necrosis of the base.


A
B
C

A Phytophthora Footrot of Rhubarb

PLATE 4

PLATE 4

A.—Phytophthora footrot of rhubarb, medium stage of natural infection.
B.—Rhubarb plant killed by artificial inoculation. The leaf stalks inoculated collapsed as in A, after which the organism entered the root, rotting it and causing the rest of the leaves to wilt.

PLATE 5

A.—Portion of a rhubarb plant recently killed by Phytophthora footrot. The organism has advanced in the roots to the points marked X.

B.—Section through portion of a rhubarb plant, showing one side killed and the other side succumbing to the advance of the fungus in the upper part of the root.


A


B

A Phytophthora Footrot of Rhubarb

PLATE 6

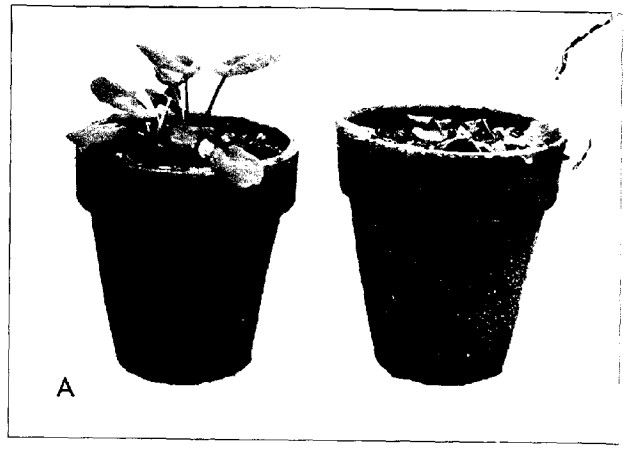


PLATE 6

A.—Seedling rhubarb in pots, the one inoculated and the other not.
B.—Rhubarb plant in the experimental field inoculated July 20, 1920, by applying the fungus from pure cultures at the wound left by pulling leaf stalks. Photograph taken six days after the inoculation. The plant was killed.

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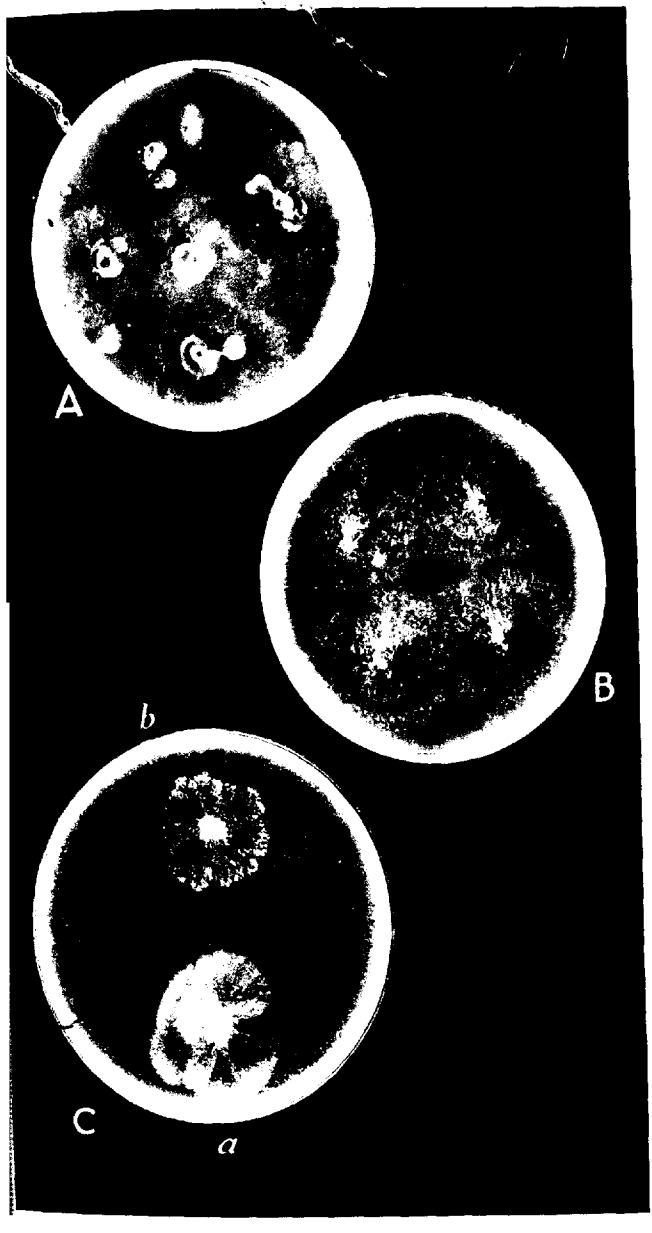
PLATE 7

Petri dish cultures of *Phytophthora parasitica* var. *rhei*.

A.—A contaminated culture.

B.—The same strain secured in pure culture by inoculating into an apple and reisolating from the advancing edge of decay.

C.—*Phytophthora parasitica* var. *rhei* (a) and *Phytophthora terrestris* Sherb. (b) compared on corn meal agar.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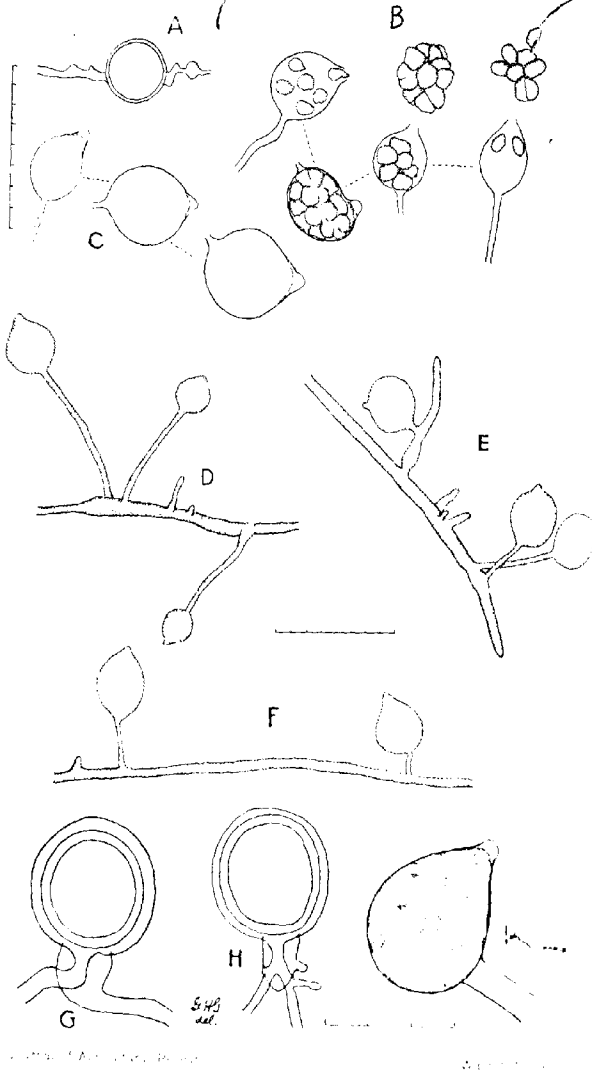
B
b
C
a


A
B
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D
E
F
G
H

PLATE 8

Camera-lucida drawings of *Phytophthora parasitica* var. *rnei*.

A.—Outline drawing of an intercalary chlamydospore, showing the thick wall, mewhat exaggerated.

B.—Various stages in zoospore emergence.

C.—Sporangia in outline, showing relation of granular protoplasmic contents to the apillae.

D, E, and F.—Sporangia as found in pure culture, attached to irregular hyphae.

G.—Oospore, showing relation of oogonium and antheridium on separate hyphae.

H.—Oospore with the antheridium apparently an outgrowth of the same hypha as at from which the oogonium developed.

I.—A sporangium drawn to show better than in C the relation of protoplasm to the apilla. Note also the thin wall of the papilla in contrast with the thick sporangium all ending abruptly.

Each scale division represents 1 100 mm.

PLATE 9

Camera-lucida drawings of *Phytophthora parasitica* var. *rhei*.

A.—Different views of zoospores killed with osmic acid fumes and stained with dilute gentian violet.

B.—Zoospores gradually coming to rest, showing enlargements at tips and disappearance of the flagellae.

C.—Irregular zoospores, one of them with four flagellae, consisting probably of two individuals not yet pulled apart. The others also were killed shortly after emergence before they had time to attain normal shape.

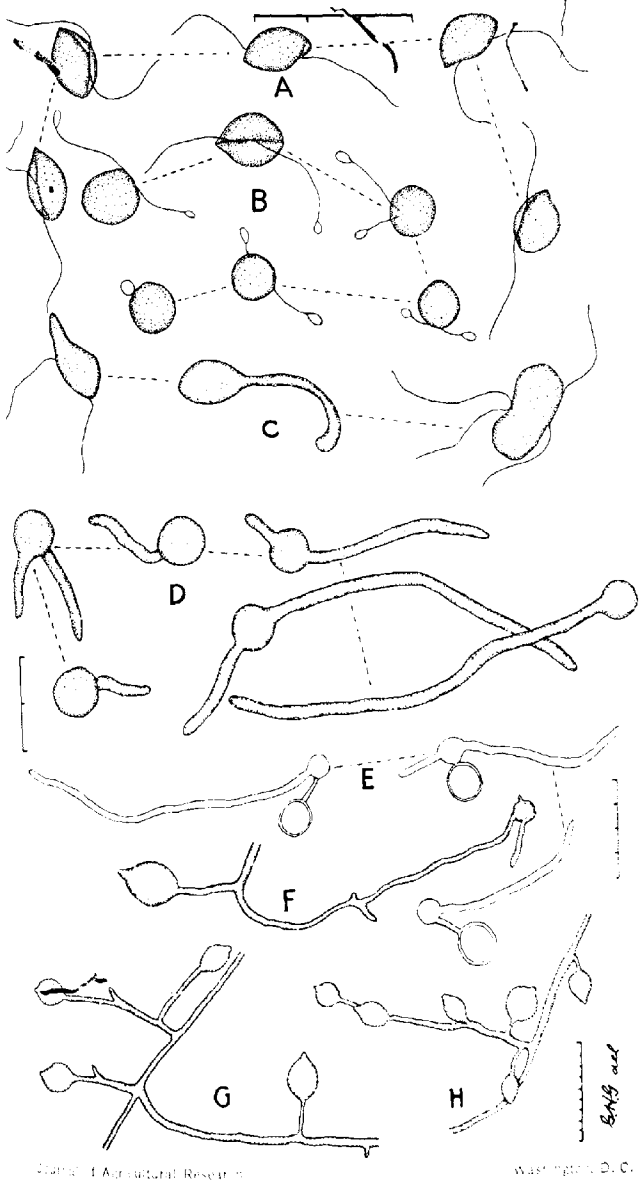
D.—Germinating zoospores in different stages.

E.—Germinated zoospores producing chlamydospores.

F.—Germinated zoospore with production of sporangium.

G, H.—Sporangium production from pure cultures.

Each scale division represents 1/100 mm.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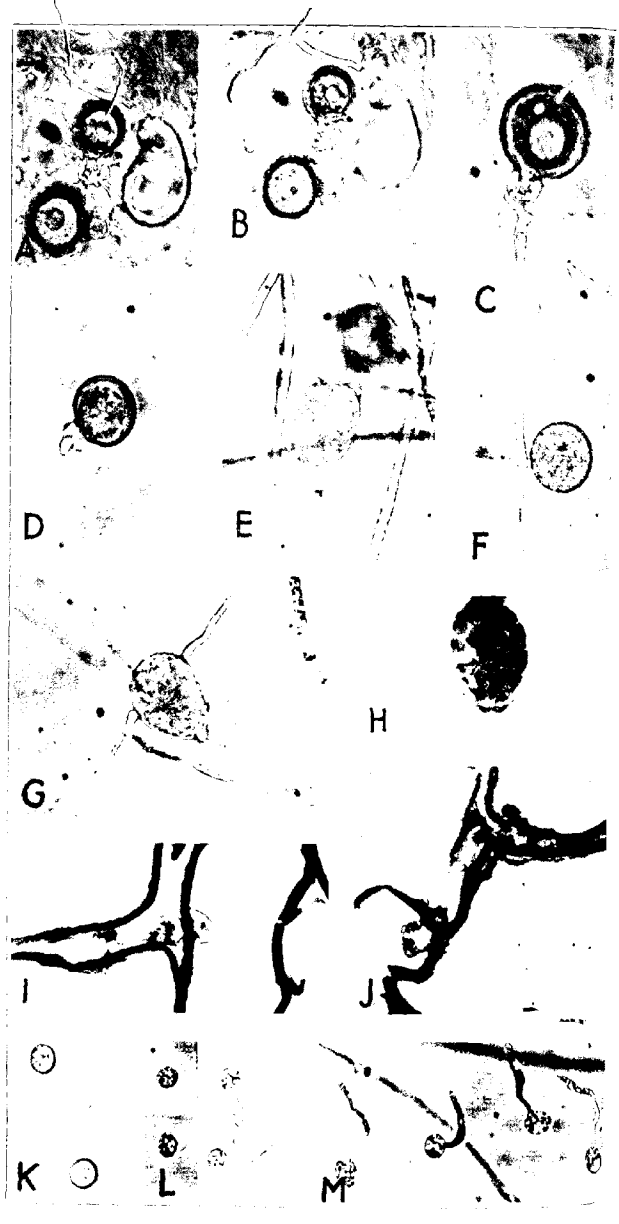
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I
J
K
L
M

PLATE 10

Photomicrographs of *Phytophthora parasitica* var. *rhei*.

A.—(Center) oospore, with focus on stalks of antheridium and oogonium.

B.—Same with focus on oospore wall.

C.—Another oospore, showing attachment of the oogonium and its relation to the ntheridium.

D.—Another oospore.

E.—A sporangium, showing granular protoplasm and papilla.

F.—A laterally attached chlamydospore.

G.—An intercalary sporangium.

H.—A sporangium killed with osmic acid fumes just before emergence of the zoo-ores would have taken place. After killing the slide was treated with chloroiodid f zinc, which stained the walls a light blue and the protoplasm yellow. Note the eavage at this stage into distinct individuals. The papilla also is clearly shown.

I, J.—Intercellular hyphae and cell wall penetration. Note in both the slender ypha at point of penetration and enlargement afterward. J shows what appears to e a typical haustorium.

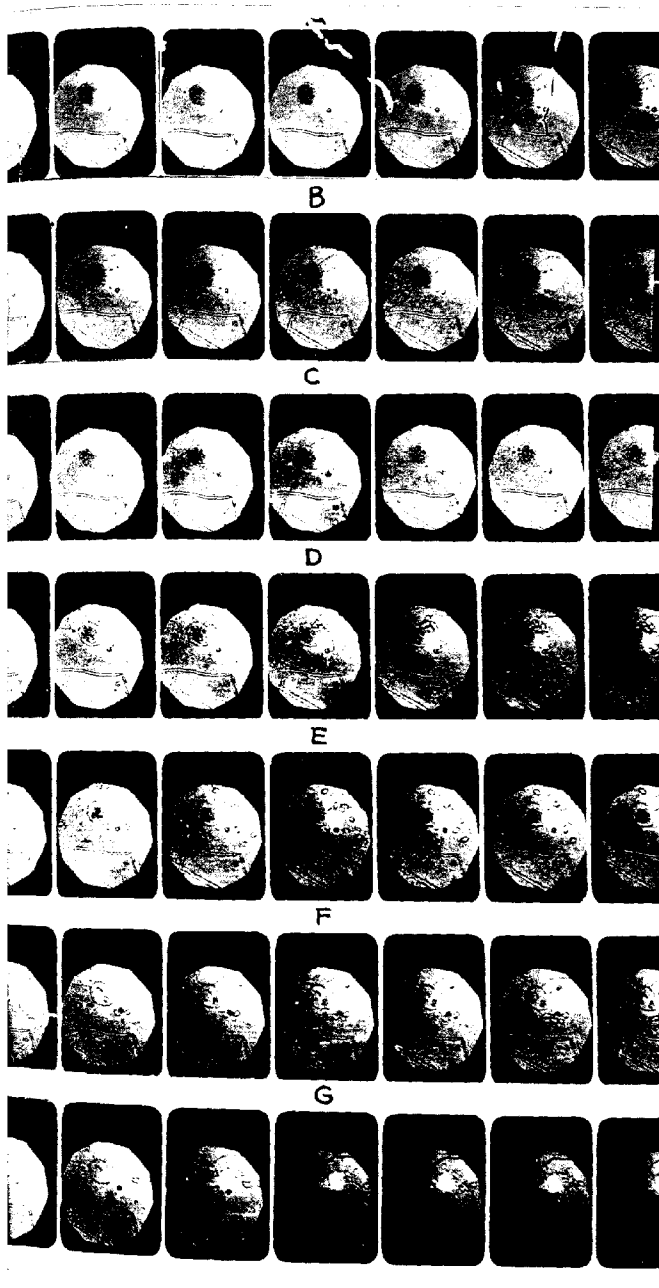
K, L, M.—Various stages in zoospore germination.

PLATE 11

Progressive stages in the emergence of zoospores of *Phytophthora parasitica* Var. *Nic.* as shown by reproductions from a motion-picture film.

Strip B follows A immediately on the film, and shows the rapid bursting outward of the papilla. The spot just in front of the sporangium is a zoospore from another sporangium. It has come to rest in a very unsatisfactory position. The glow of light just at the opening of the sporangium is another unsatisfactory feature which could probably be eliminated with more experience.

Strips C to G were taken from widely separated intervals in the film, and show subsequent stages in the escape of zoospores.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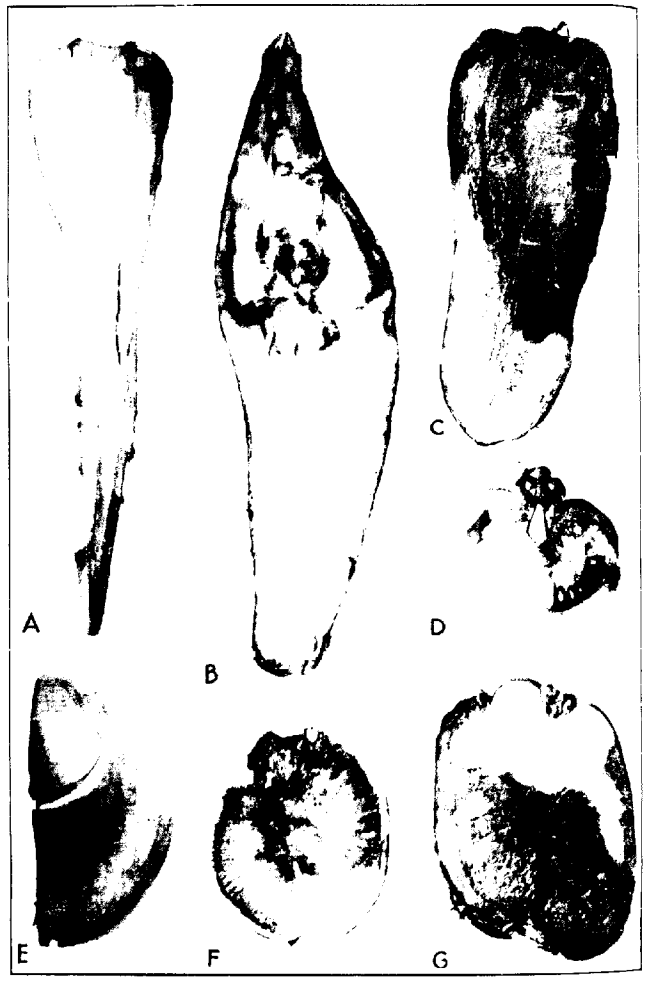
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G

PLATE 12

Effect of *Phytophthora parasitica* var. *rhei*, on different plants, the darker area in each ase being the region penetrated by the hyphae after three days' incubation.

A.—Parsnip.
B.—Sweet potato.
C.—Carrot.
D.—Tomato.
E.—Stayman Winesap apple.
F.—Turnip.
G.—Potato.

AND DROWN, A CHLOROSIS OF TOBACCO DUE TO MAGNESIUM DEFICIENCY, AND THE RELATION OF SULPHATES AND CHLORIDS OF POTASSIUM TO THE DISEASE[1]

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DESCRIPTION OF SAND DROWN AND ITS EFFECTS

The disease of tobacco which has received the name "sand drown" ears no essential relation to true drowning from excessive moisture in he soil. On the contrary, as indicated in the name itself, the disease s likely to be most severe on deep, sandy soils which never become ater-logged. The trouble is aggravated by heavy rainfall, as suggested y the word "drown;" but, as will be subsequently developed, the ffects of the rainfall are chiefly those of leaching rather than of the resence of the water itself. The dominant symptom is a characteristic ype of chlorosis in which the yellow as well as the green pigments of hlorophyll are affected. Thus, in the affected tissue the color is a ull, very pale yellow, and in extreme cases almost pure white. This olor is readily distinguished from the clear lemon or orange color com- ionly observed in the healthy leaf which has been exposed to darkness or a brief period of time or has undergone partial curing. The chlorosis egins on the lowermost leaves and at the tips of the leaves affected. In dvanced stages practically the whole area of the leaf is involved and early all the leaves of the plant may be affected. The advance of the hlorosis is from the tip and the margin toward the base and the central one of the leaf. Successive stages in the progress of the bleaching effect n the leaf are shown in Plates 2 and 3. Sand drown rarely appears in he field till the plants have attained considerable size, more commonly fter topping, so that the leaves usually attain the normal size and shape, s is indicated in Plate 1. The veins of the leaf, as well as the tissue mmediately adjoining the veins, show a tendency to retain the normal green color long after the remaining leaf tissue has become blanched.

The disease is scarcely less evident in the cured leaves than in the green leaves in the field. In the flue-cured district normal leaves when properly cured have a bright lemon to orange color, and in other districts the colors range through various shades of brown with either a

[1] Accepted for publication Sept. 1, 1921. Cooperative investigations of the Office of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the North Carolina Department of Agriculture.

reddish or a greenish cast. On the other hand, the leaves or the por tions of leaves affected with sand drown show after curing a dull, fade appearance similar to that observed in the diseased leaves in the field Since color is of great importance in determining the commercial valu of most types of tobacco, it is easily seen that sand drown ma greatly lower the market price of the affected leaves. This applie with special force to the sale of cigar-wrapper leaf. Moreover, san drown greatly reduces the weight of the cured product; in fact th affected parts of the leaf are remarkably thin after having been cure For example, in a test with flue-cured tobacco the normal leaf wa found to be 45 per cent thicker and 65 per cent heavier per unit are than the diseased leaf. As would be expected, the tissue of disease leaves is dry and lifeless. Thus, sand drown affects unfavorably th color, weight, body, and elasticity of tobacco leaf.

OCCURRENCE OF SAND DROWN

The disease first came under observation about 1912 at the Tobacc Station of the North Carolina Department of Agriculture located a Oxford, in connection with the cooperative investigations in progress a that point. The same trouble also was seen on some of the farms o tobacco growers in the vicinity. It has appeared each year on th tobacco grown at the Oxford station, but it soon became apparent tha weather conditions and the kinds and quantities of fertilizers applie affect in a marked degree the appearance and course of the disease. I general, it may be said that with approximately normal rainfall and th use of fertilizers of the grades and the quantities that have been mos widely employed in tobacco culture sand drown is not commonly seen in sufficiently severe form to attract attention or to cause serious los On the other hand, the malady is likely to be widespread in seasons o heavy rainfall in those sections in which the dominant soil types are distinctly sandy in character. For example, this type of chlorosis i frequently seen on the more sandy tobacco soils of the Connecticu Valley in excessively wet seasons. As will be pointed out, the use o certain fertilizer salts will lead to symptoms of sand drown even in sea sons of normal rainfall and on the heavier types of soil, although i remains to be determined just how generally this action may be observed under varied soil and weather conditions. It is possible, also, tha material injury to the color and other properties of the cured leaf may result from mild forms of the trouble which would not be clearly recog nizable in the field. In pot cultures sand drown can be easily induced and cured at will.

RELATION OF SULPHATES AND CHLORIDS TO SAND DROWN

As a feature of the cooperative investigations at the Oxford (N. C Tobacco Station, fertilizer plot tests have been in progress for several years in which various forms and rates of application of nitrogen, phos phoric acid, and potash are compared. The soil is classed as Durham sandy loam and is not fertile. Sand drown has frequently appeared on the test plots. On most of the plots the tobacco has been fertilized with sulphate of potash in combination with other materials, and it was observed that increase in the rate of application of the sulphate clearly increased the severity of sand drown in the tobacco. On plots receiving

uriate of potash in place of the sulphate, other conditions being the me, there was a marked decrease in the amount of disease and an ›preciable increase in the size attained by the plants. In a second set plot tests, in which a more detailed comparison between the muriate id sulphate of potash was made, the striking effect of the sulphate in ducing or aggravating the disease was fully confirmed. In the first t of plots the standard rate of application of the potash salts was 80 ›unds per acre actual potash (K_2O), while certain plots received 40 and ›o pounds per acre. In the second series of plots the rates of application ère 12, 24, 36, and 80 pounds per acre. In the first series the normal ›plication of phosphoric acid was 64 pounds per acre in the form of 5 per cent acid phosphate, and the normal application of nitrogen was 2 pounds, various forms being used. Even the smallest applications f potassium sulphate produced marked effects in increasing sand drown. t is obvious that these quantities are much too small to produce injury y unduly increasing the concentration of the soil solution, and, moreover, ›rresponding quantities of potassium chlorid have shown no injurious ›tion of any sort. The peculiar action of the sulphate, contrasting so ıarply with the chlorid, seemed perplexing, for, while in recent years ›nsiderable stress has been placed on the necessity of an adequate supply f sulphur for the plant, no case has been previously recorded, so far s known, in which small quantities of sulphates caused serious injury nder field conditions.

A series of single-row tests was carried out at the Oxford station in hich sodium chlorid, sodium carbonate, and calcium carbonate were sed, in addition to the normal application of potassium sulphate, dried lood, and phosphorous in the form of so-called precipitated bone, a nely divided dicalcic phosphate. The common salt and sodium car-onate were used at the rate of 200 pounds and the calcium carbonate t the rate of 1,000 pounds per acre. The sodium carbonate was not ffective in preventing sand drown, and the calcium carbonate was only artially effective, neither of these materials causing appreciable increase ı growth of the tobacco. On the other hand, the sodium chlorid ›rdinary commercial "salt," sold in bulk) probably increased the growth f the tobacco by fully 100 per cent, and there was no sand drown. The ext season another series of row tests was carried out in which chemi-ally equivalent quantities of the chlorids and sulphates of sodium, alcium, and magnesium were used, in addition to a standard application f 100 pounds per acre each of ammonium nitrate and monopotassium hosphate. All salts were approximately chemically pure, and the uantities used were on the basis of 100 pounds sulphate (SO_4) per acre r the equivalent quantity of chlorin. In this case the sodium chlorid nd the calcium chlorid were without effect in controlling the sand drown. loth the sulphate and the chlorid of magnesium, however, showed a ıarked effect in preventing the disease; and this observation first gave he clue to the situation, as will be shown in subsequent paragraphs.

RELATION OF ORGANIC MATTER TO SAND DROWN

On the fertilizer test plots at Oxford several different sources of nitro-en were used—namely, nitrate of soda, ammonium sulphate, dried lood, and cottonseed meal. These materials were applied at rates to ırnish about 32 pounds nitrogen per acre. the quantity of cottonseed

meal employed being 500 pounds. It was quickly seen that the cottonseed meal showed a decided tendency to reduce or to delay the symptoms of sand drown while ammonium sulphate accentuated the trouble. There was no notable difference between the action of nitrate of soda and that of dried blood. Once more the injurious action of sulphates is in evidence, but, in addition, it is clear that only 500 pounds per acre of organic matter in the form of cottonseed meal exercises some preventive action against the disease. Apparently there is some similarity in the action of certain chlorids and that of cottonseed meal calling for explanation. Different rates of application of nitrogen in the form of dried blood produced no notable effect on the disease, so that neither the form nor the quantity of nitrogen in the fertilizer can be considered as being of primary importance.

Another series of plot tests was conducted for several years at Oxford dealing with the fertilizing value of tobacco stalks and stems (leaf midrib) and barn manure for tobacco. For purposes of comparison the normal mixture of sulphate of potash, dried blood, and acid phosphate also was included in this series. The tobacco stems were applied at rates ranging from 1,000 to 2,600 pounds per acre, supplemented with sufficient quantities of dried blood and acid phosphate to furnish the normal application of 32 pounds nitrogen and 64 pounds phosphoric acid per acre. The tobacco stalks were applied at the rate of 2,700 pounds per acre, also supplemented with acid phosphate. The manure was used alone, in combination with sufficient acid phosphate and sulphate of potash to furnish the normal formula, and in combination with hardwood ashes as a source of potash. The rate of application of the manure was 8,000 pounds per acre. As regards sand drown, the outstanding feature of these tests was that there were no symptoms of the disease except on the plots receiving only the mixture of dried blood, acid phosphate, and sulphate of potash. Here again is distinct evidence that vegetable forms of organic matter tend to prevent the development of sand drown.

BIOCHEMICAL STUDIES OF SAND DROWN

Because of the marked difference between the chlorid and the sulphate of potassium in their action on the tobacco plants, especially with regard to sand drown, experiments were undertaken for the purpose of comparing the effects of the two salts on the internal processes of the plant. A series of observations was made on the relative osmotic concentration of the expressed, filtered saps from the frozen tissues of the leaf after the midrib had been removed, the material for study being taken from the two sets of fertilizer test plots at Oxford previously referred to. The samples were taken at intervals of two to four days, beginning shortly after the plants had been topped, that is, when the plants had reached the normal stage for flowering. A single leaf near the middle of the plant was collected from a representative plant of each plot, and the same plants were used as sources of material throughout the tests. The leaves were collected in each case about 9 o'clock in the morning. In all cases the tobacco plants on the control plots receiving no potash showed distinct symptoms of severe potash hunger. The data obtained are summarized in Table I. The osmotic concentrations, in atmospheres, were calculated in the usual way from the observed lowering of the freezing point.

TABLE I.—*Osmotic concentration of the sap of tobacco leaves and the ash of the sap, as affected by the chlorid and the sulphate of potassium*

Fertilizer treatment.[a]	Osmotic concentration of sap (in atmospheres).					Osmotic concentration of ash of sap (in atmospheres).	
	July 22.	July 24.	July 26.	July 30.	Average.	July 22.	July 24.
eneral fertilizer test plots:							
N+P	9.346	8.396	9.093	8.913	8.937	2.929	1.869
N+P+80 pounds K_2O, as sulphate	.....	9.021	9.057	7.878	8.652	.....	6.012
N+P+160 pounds K_2O, as sulphate	11.060	10.530	9.418	9.875	10.220	6.855	7.349
P+80 pounds K_2O, as sulphate	8.937	8.949	8.576	7.987	8.612	5.940	6.266
N+80 pounds K_2O, as sulphate	8.540	8.492	8.215	7.951	8.299	5.013	4.917
Average moisture percentage of soil of plots	7.0	5.5	7.25	9.0			

Fertilizer treatment.[a]	Osmotic concentration of sap (in atmospheres).					Osmotic concentration of ash of sap (in atmospheres).	
	July 20.	July 23.	July 25.	July 29.	Average.	July 20.	July 23.
pecial potash test plots:							
N+P	7.734	8.191	8.504	8.071	8.125	0.917	1.748
N+P+24 pounds K_2O, as chloride	9.574	8.720	10.240	8.600	9.283	3.086	2.868
N+P+24 pounds K_2O, as sulphate	8.768	8.780	9.298	8.107	8.753	2.712	3.351
N+P+80 pounds K_2O, as chlorid	10.070	10.670	10.760	9.959	10.360	7.710	8.191
N+P+80 pounds K_2O, as sulphate	9.635	8.805	10.280	8.528	9.312	5.182	4.929
Average moisture percentage of soil of plots	8.0	5.5	9.0	9.0			

[a] N=32 pounds nitrogen per acre, in form of dried blood. P=64 pounds phosphoric acid per acre, in orm of acid phosphate in first series and precipitated bone in second series

Concerning weather conditions during the period of observation it may be stated that cloudy weather, with showers, prevailed July 17, 19, 24, 27, and 31, with sunshine on all other days, except for a few thunder showers of brief duration. Rainfall of consequence occurred only as follows: July 17, .33 inches; July 24, 1.55 inches; July 27, 1.37 inches; July 30, 0.82 inch. The soil, which is a light, sandy loam, has a low water-holding capacity, and there was a wide range in degree of saturation during the period of study. The osmotic concentration of the sap of the plant, however, does not appear to be definitely correlated with the water content of the soil. In general, increased application of potash salts to the soil increased the osmotic concentration of the sap and the chlorid was decidedly more effective than the sulphate in this respect.

The mineral component of the osmotically active material of the sap was determined by evaporating the sap, igniting the residue, and restoring the original volume with distilled water. The data in Table I clearly show that where no potash was applied to the soil much the greater proportion of the osmotic concentration represents soluble organic material, probably carbohydrate, whereas with increasing quantities of potash salts applied to the soil this relationship is reversed, the mineral component becoming the more important. The chlorid, moreover, contributes more to the osmotic concentration of the sap than does the sulphate. These observations seem to be in line with the view which has long been held that lack of an adequate supply of potash tends to cause congestion or accumulation of carbohydrate in the tissues of the leaf.

The saps (in 10 cc. portions) from the general fertilizer test plots collected on July 24 and those from the special potash plots collected on July 23 were employed in studying the quantity and character of the ash contained in the sap, as influenced by the sulphate and the chlorid of potassium. The total ash was determined by ignition and the soluble ash by extracting the ignition residue with successive portions of hot distilled water aggregating a volume of 100 cc. The alkalinity of the soluble and insoluble portions of the ash was determined by titration, using methyl orange as indicator. The calcium in the insoluble ash was determined gravimetrically. The results are presented in Table II.

TABLE II.—*Character of the ash of tobacco sap as affected by the chlorid and the sulphate of potassium*

Fertilizer treatment of tobacco.	Total ash.	Soluble ash.	Insoluble ash.	Alkalinity of soluble ash.[a]	Alkalinity of insoluble ash.[a]	CaO in insoluble ash.
	Gr.	*Gr.*	*Gr.*			*Gr.*
Regular fertilizer test plots:						
N+P	0.1004	0.0538	0.1156	0.6	22.4	.....
N+P+80 pounds K_2O as sulphate	.1816	.1320	.0496	7.1	10.5	.....
N+P+160 pounds K_2O as sulphate	.2219	.1748	.0471	11.4	0.4	.....
N+80 pounds K_2O as sulphate	.1497	.1167	.0330	11.7	7.2	.....
P+80 pounds K_2O as sulphate	.1801	.1531	.0270	10.3	5.0	.....
Special potash test plots:						
N+P	.1007	.0450	.1549	4.1	32.6	0.090
N+P+24 pounds K_2O as chlorid	.1528	.0574	.0954	.7	20.1	.055
N+P+24 pounds K_2O as sulphate	.1822	.0707	.1115	7.3	23.5	.061
N+P+80 pound K_2O as chlorid	.1505	.1228	.0367	.6	8.6	.020
N+P+80 pounds K_2O as sulphate	.1661	.1202	.0459	10.7	10.3	.028

[a] Expressed in cubic centimeters of *N*/10 acid required for titration.

Both the chlorid and the sulphate of potash produce marked increases in the soluble portion of the ash of the sap and equally as large decreases in the insoluble portion. There is no marked difference in this respect in the action of the two salts, perhaps indicating that approximately equal quantities of potash are absorbed by the plant from these two sources when the rates of application to the soil are equal. That the effects of the two salts on the metabolism of the plant are different, however, is clearly shown by the alkalinity of the soluble and insoluble

portions of the ash. Roughly, the alkalinity of the soluble ash is a measure of the potash in combination with citric and malic acids in the sap, while the alkalinity of the insoluble portion represents the calcium and magnesium salts of these acids existing in solution in the sap. Both the sulphate and the chlorid greatly reduce the alkalinity of the insoluble ash, that is, the content of the sap in calcium and magnesium citrates and malates. The most striking effect, however, is the great increase in the sap content of potassium salts of citric and malic acids caused by the sulphate, as indicated by the content of soluble carbonate in the ash. The chlorid, on the other hand, is wholly ineffective in this respect. This difference in the effect of the sulphate and the chlorid on the content of soluble salts of organic acids is of practical importance, for it is known that the potassium salts of citric and malic acids improve the combustibility of tobacco (*1*).[2] On the other hand, the acid calcium salts of these acids, which largely make up the grain particles of the cured tobacco leaf (*5*), injure the combustibility, especially if the grain material is not definitely aggregated. If the organic acids are intermediate products in the respiratory combustion of carbohydrate in the plant, it appears that in some way the oxidative processes are less complete in the presence of the sulphate than when the potassium is supplied as chlorid. It is seen from Table II that addition of the potash salts to the soil lowered the calcium content of the plant sap.

It seemed possible that the unfavorable action of potassium sulphate on the vigor of the tobacco plant might be due to injurious increase in hydrogen-ion concentration of the cell sap. To test this matter leaves were collected at intervals, in the manner already described, from the special potash test plots and from other specially treated plots, using six plants on each plot for material. The leaf material was not subjected to freezing but was ground in a well-tinned meat chopper and the expressed sap was passed through muslin. The hydrogen-ion concentration of the saps thus prepared was determined electrometrically at room temperature according to the usual procedure. The results are presented in Table III.

TABLE III.—*Hydrogen-ion concentration of cell sap as affected by the sulphate and the chlorid of potassium and other salts*

Fertilizer treatment of tobacco.	Hydrogen-ion concentration of sap collected at stated intervals.				
	July 18.	July 23.	July 28.	Aug. 5.	Average.
Special potash test plots:	P_H	P_H	P_H	P_H	P_H
Control	5.47	5.40	5.26	5.26	5.35
36 pounds K_2O per acre as sulphate	5.61	5.05	5.47	5.43	5.54
36 pounds K_2O per acre as chlorid	5.43	5.33	5.29	5.28	5.33
80 pounds K_2O per acre as sulphate	5.50	5.03	5.52	5.46	5.53
80 pounds K_2O per acre as chlorid	5.04	5.50	5.46	5.29	5.48

[2] Reference is made by number (italic) to "Literature cited," p. 11.

TABLE III.—*Hydrogen-ion concentration of cell sap as affected by the sulphate and the chlorid of potassium and other salts*—Continued

Fertilizer treatment of tobacco.	Hydrogen-ion concentration of sap collected at stated intervals.			
	July 21.	July 30.	Aug. 6.	Average.
Special row tests:	P_H	P_H	P_H	P_H
Control	5.60	5.43	5.41	5.48
200 pounds NaCl per acre	5.85	5.63	5.45	5.64
Control		5.60		
200 pounds Na_2Co_3 per acre		5.62		
Control	5.70	5.86		
1,000 pounds $CaCo_3$ per acre	5.95	5.85		

The complete fertilizer treatments of the plots used in the observations on hydrogen-ion concentration are given on pages 29 and 31. The data given in Table III show that there is a progressive increase in hydrogen-ion concentration of the leaf sap as the leaf advances toward maturity. This progressive increase in active acidity is markedly retarded by the sulphate of potassium and to a lesser degree by heavier applications of potassium and sodium chlorids. Neither calcium carbonate nor sodium carbonate produced any marked effect. It is clear that the sulphate of potassium did not increase the active acidity and, in fact, it caused a considerable decrease in acidity. In these tests the leaves from plots treated with chlorids were free from sand drown, while the others, and especially those from the plots receiving sulphates, showed evidences of the disease. Thus, while sulphates may promote the development of a pathological chlorosis they actually retard the normal yellowing of the leaf which is associated with ripening.

The total calcium and the water-soluble sulphate (SO_4) were determined in dried leaves from the plot treated with common salt and the control plot, as well as from the plots receiving 80 pounds potash per acre as sulphate and chlorid, respectively. The results are shown in Table IV.

TABLE IV.—*Influence of sulphates and chlorids on total calcium and water-soluble sulphate in the dried leaf*

Fertilizer treatment of tobacco.	Total ash in leaves.	Total calcium in leaves.	Water-soluble sulphate (SO_4) in leaves.
	Per cent.	*Per cent.*	*Per cent.*
Control	13.00	3.40	1.00
200 pounds NaCl per acre	12.09	1.74	.07
80 pounds K_2O per acre as chlorid	8.89	1.40	.35
80 pounds K_2O per acre as sulphate	10.50	1.30	1.12

It seems likely that the greatly increased growth of the plant resulting from the use of the sodium chlorid is largely responsible for the decrease in percentage content of calcium and soluble sulphate. The calcium content is the same in the plots treated with the sulphate and the chlorid

of potassium, and since sand drown was present on the plants receiving the sulphate the disease can not be due to calcium deficiency. The increased content of soluble sulphate, however, is undoubtedly significant.

No evidence of soluble oxalates could be found in the sap from leaves affected with sand drown.

MAGNESIUM DEFICIENCY THE CAUSE OF SAND DROWN

As previously stated, the effectiveness of magnesium sulphate in preventing sand drown in field tests at Oxford while other sulphates greatly aggravated the trouble suggested that lack of an adequate supply of magnesium might be the cause of the trouble. Pot tests soon proved this to be the case. The cultures were conducted in 12-quart galvanized-iron buckets with perforated bottoms, using sassafras fine sandy loam soil from Prince Georges County, Md., and in some cases the Durham sandy loam soil from the Oxford (N. C.) Tobacco Station. As a complete nutrient solution for the cultures a mixture of 10 gm. sodium nitrate, 5 gm. monocalcium phosphate, 5 gm. potassium sulphate and 5 gm. magnesium sulphate was dissolved in 10 liters distilled water. In some cases potassium chlorid was substituted for the sulphate and ammonium nitrate was used in place of the sodium salt. For one series in each test the magnesium sulphate was omitted from the nutrient solution. To accentuate the effect of omitting magnesium the plan was adopted of watering the plants with the nutrient solution instead of distilled water, the solutions being added in considerable excess so as to cause a leaching effect. This plan proved to be quite successful. Where magnesium was omitted from the solution sand drown quickly appeared. A typical case in the advanced stage is shown in Plate 4. It will be seen that the leaves have lost nearly all color and hang limp, the cells having lost their turgidity. It is to be noted, however, that seed formation was successfully accomplished and the chlorotic leaf tissue remains alive for many days. Under favorable conditions the disease may be induced by applying the fertilizer salts (with magnesium omitted) to the soil before transplanting and avoiding any leaching action in watering the plants, as is shown in Plate 5. Naturally, the symptoms are less severe than when leaching is employed. When the disease has not progressed too far it is readily cured by addition of magnesium to the soil. A case of this sort is shown in Plate 6. The plant at the right, affected with sand drown in severe form, to which magnesium sulphate had been subsequently added was rapidly recovering at time of photographing. Thus, while soluble sulphates generally will greatly aggravate sand drown, sulphate of magnesium both prevents and cures the malady. It seems proper, therefore, to consider lack of an adequate supply of magnesia as the primary cause, whatever may be the internal processes involved. On the other hand, the importance of an increased supply of soluble sulphate as an inciting factor should not be overlooked. In other words, the ratio of magnesia to soluble sulphate seems to be of prime importance.

SAND DROWN CONTRASTED WITH OTHER TYPES OF CHLOROSIS

The tobacco plant with its extensive foliar expanse is well adapted to the study of pathological symptoms such as those characteristic of sand drown; and the plant, in fact, is subject to several types of chlorosis. Of special importance in this connection is the chlorosis due to potash hunger. In typical cases, the symptoms of potash hunger are readily

distinguished from those of sand drown, though the two diseases may be confused in casual examination. In both cases the chlorosis begins at the tips of the leaves and on the margins, and the lower leaves are affected first. In extreme cases of sand drown there may be some distortion of the leaf margin, but ordinarily the surface is smooth and the leaf attains full size. In potash hunger the leaf is puckered and presents an uneven surface because of the difference in rate of growth of the veins and the leaf web. Arrested growth of the periphery causes a characteristic curving downward of the margin, and, particularly, the tip of the leaf. The farmer speaks of the leaf as being "rim bound." Chlorosis due to potash hunger is promptly followed by the appearance of small dead spots in the affected portions of the leaf. The initial stages of this spotting are shown in Plate 7. As the malady progresses large areas of the leaf die, especially along the margins which frequently become ragged and torn. This condition is popularly spoken of as "rimfire." This localized dying of the leaf tissue in potash hunger is an important aid in distinguishing the trouble from sand drown. Finally, the destruction of the green color of the leaf in potash hunger gives rise to a dull yellow, with a bronze or copper overcast, whereas sand drown results in a very light yellow or cream color. In severe potash hunger the green portion of the leaf shows a dark "muddy," bluish green shade, while in sand drown the non-chlorotic portion of the leaf shows the normal green color.

In sulphur deficiency the entire leaf, including veins and midrib, as well as the stem of the plant, is uniformly affected, the color of the whole being a light shade of green. This color is frequently shown when muriate of potash is used instead of the sulphate as fertilizer. When both sulphur and magnesia are deficient the entire plant becomes more distinctly chlorotic, the color being suggestive of that characteristic of the White Burley variety of tobacco as it approaches maturity. As is well known, a limited nitrogen supply also results in a plant with leaves of a light shade of green. In the disease known as frenching there is a partial chlorosis of the leaves, but there are also morphological changes, the size and especially the width of the leaf being reduced through failure of the leaf lamina to expand normally. In the infectious chlorosis called mosaic there is a characteristic mottling distributed rather uniformly over the leaf and appearing only in growing tissues; hence it is easily distinguished from sand drown.

MAGNESIA AS A FACTOR IN PRACTICAL FERTILIZER USAGE

It has been recognized for many years that magnesium is one of the indispensable elements of plant food, and Willstätter (*7*) has demonstrated that it is an essential constituent of chlorophyll. A considerable number of experiments have been made concerning the fertilizing value of magnesium compounds, the results of which are more or less contradictory. Few of these tests, however, have been so planned as to throw light on the present problem, and in most cases the action of magnesium salts has been regarded as being indirect, resulting in the liberation in the soil of other plant food elements (*2*). In pot experiments with one of two soil types studied, Wheeler and Hartwell (*6*) obtained increased growth of barley and rye by the use of magnesium sulphate and chlorid, and it is suggested that the favorable effect was due, at least in part, to a direct nutrient action of the magnesium. Recently A. Jacob (*3*) has called attention to favorable results obtained by the German Kali Syndicate in the use of potash salts containing magnesia as fertilizer for pota-

toes. Considerable attention also has been given to the toxic effects of magnesia compounds when present in excess, especially since Loew developed his well-known theory of optimum lime-magnesia ratios for crop plants (*4*).

It seems clear that on many soils it is essential for normal development of the tobacco plant that the fertilizer carry a certain minimum quantity of magnesia, especially when the fertilizer contains soluble sulphates. Preliminary observations indicate that corn and cotton and probably other crops have similar requirements. The exact needs of the tobacco crop will require considerable study, since both the yield and the quality of the product are involved. The combustibility of tobacco is affected adversely by salts of magnesia, and it appears that the moisture-holding properties, and therefore the elasticity, of the leaf are influenced by the magnesia supply. The highly injurious effect of magnesium deficiency on the color of the leaf has already been discussed. Hence, it seems likely that any considerable excess of magnesia, as well as a deficiency, must be avoided for best results.

Detailed data of plot yields with tobacco in different localities for a period of years comparing the action of sulphate and chlorid of potash, which involve the effects of magnesium deficiency, are reserved for publication elsewhere. It will suffice to present here the results obtained at Oxford, N. C., in 1920, as illustrating the striking effect of magnesium deficiency on the market value of the crop. The detailed fertilizer treatment of these plots has already been described. One-half of each plot received ground limestone containing magnesia applied broadcast in the fall of 1919 at the rate of 1 ton per acre. The yield and value of the tobacco on each plot are shown in Table V.

TABLE V.—*Yield and value per acre of tobacco receiving sulphate and muriate of potash, with and without addition of ground limestone. Oxford, N. C., 1920*

Potash (K_2O) added as fertilizer	Yield of tobacco		Value of tobacco	
	Without lime	With lime	Without lime	With lime
	Pounds	*Pounds*		
None	330	480	$27	$38
12 pounds per acre, as sulphate	260	660	33	105
12 pounds per acre, as muriate	570	640	67	126
24 pounds per acre, as sulphate	480	620	47	93
24 pounds per acre, as muriate	620	820	107	147
36 pounds per acre, as sulphate	660	640	88	60
36 pounds per acre, as muriate	780	780	150	117
80 pounds per acre, as sulphate	700	580	78	167
80 pounds per acre, as muriate	800	620	115	218

Considering only the treatments without lime, it is seen that the muriate gives far better results than the sulphate; and the difference becomes most striking with the intermediate rates of application. The injury in quality of the tobacco from the use of sulphate is proportionately greater than the reduction in yield as compared with the muriate. Evidence has already been given that addition of magnesia fully overcomes the unfa-

vorable action of the sulphate. Results fully as remarkable as the foregoing have been obtained by Mr. E. H. Mathewson, of this office, in similar cooperative tests at Reidsville, N. C.; and the experiments elsewhere indicate that the data will apply to the tobacco crop more or less generally.

Muriate of potash tends to impair the combustibility of tobacco and in some cases gives less desirable colors. It seems desirable, therefore, to use the sulphate of potash in combination with sulphate of magnesia. Tests have shown that low-grade potash salts carrying more or less magnesia, such as Kainit and double manure salts, eliminate sand drown and produce much better growth than high-grade sulphate. It has been found, in fact, that both muriate and sulphate of potash in relatively pure form but still containing very small percentages of magnesium salts, such as some of the "high-grade" imported potash salts, are less likely to cause sand drown than muriate and sulphate of exceptional purity. It seems clear that very pure forms of potash salts should not be used as fertilizer for tobacco, and possibly not for other crops, unless supplemented with magnesia salts or materials containing magnesia. In the absence of more exact data double manure salts, which are essentially mixtures of sulphate of potash and sulphate of magnesia, would seem to be a desirable source of potash for tobacco. Wood ashes, cottonseed-hull ashes, and other forms of potash of vegetable origin also should be satisfactory so far as concerns magnesia.

It is to be noted that in actual fertilizer practice high-grade sulphate of potash has not been used very extensively in this country, and even when employed it is nearly always in combination with other fertilizer materials which contain magnesia. It is believed that the favorable action of cottonseed meal, tobacco stems and stalks, and barn manure in preventing sand drown, which has been previously discussed, is due to the magnesia supplied by these materials. Cottonseed meal contains about 1 per cent magnesia, and this offers a possible explanation of the marked preference for this fertilizer material shown by tobacco growers of the Connecticut Valley where color of the cured leaf is of so much importance. It may be that vegetable material of this character through gradual decay in the soil furnishes a more or less continuous source of magnesia in suitable quantities through the growing season. Thus, it seems likely that one of the functions of barn manure is to furnish magnesia in suitable quantity and form for plant growth. The action of common salt in preventing sand drown, which has been referred to, probably is due in part to the small percentage of magnesia which it usually contains, but it is also likely that salt liberates magnesia when applied to the soil.

LIME IN RELATION TO SAND DROWN

While limestones vary widely in their content of magnesium, it rarely happens in practice that limestone or burnt lime available for agricultural uses is entirely free from this element. The available evidence all goes to show that the quantity of magnesium required to prevent the sand-drown type of chlorosis is small, possibly less than 20 pounds per acre. In general, therefore, it is to be expected that liming the soil even with fairly pure limestone or its products would prevent sand drown, keeping in mind, of course, that the magnesia thus supplied would be less soluble than that furnished by most of the impure potash salts.

The data given in Table V plainly show that liming is decidedly beneficial in overcoming the tendency of sulphate of potash to cause

sand drown, and heavier applications probably would further increase the effectiveness of both the sulphate and the chlorid of potassium by removing magnesium deficiency as a limiting factor. It is of interest to note that Wheeler and Hartwell (6) found a marked increase in content of magnesium but not of calcium in both red clover and common sorrel (Rumex) grown on soil which had been limed. The value of liming in overcoming sand drown has been confirmed in other tests involving the use of various potash salts containing different percentages of magnesia. In all cases sand drown associated with the use of the purer potash salts was entirely prevented by applying dolomitic limestone. Thus, to the various functions in improving soil productiveness which have been ascribed to liming must be added that of preventing or correcting magnesium deficiency. Again, it has been shown that one of the harmful effects which may result from the use of ammonium sulphate as fertilizer without liming is to cause a deficiency of magnesium, and this injurious effect may be remedied by use of magnesium salts as well as by liming. It is to be noted that the value of limestone in supplying small quantities of magnesia has little or no connection with the theory of optimum lime-magnesia ratios.

NEW METHOD FOR CONDUCTING POT CULTURES IN THE STUDY OF FERTILIZER PROBLEMS

It is well known that it is difficult in most cases to harmonize the results of pot tests conducted under accurately controlled conditions with results obtained in the field. Experience obtained in the study of sand drown has emphasized two important particulars in which the two types of experimentation usually differ. In the first place, chemically pure salts are generally used in pots cultures while in the field pure fertilizer salts are practically never used. In the second place, in conducting pot tests precautions are usually taken against any loss of material through drainage, while in the field more or less leaching invariably occurs. By common consent investigators and farmers alike constantly speak only of the three elements, nitrogen, phosphorus, and potassium (with occasional reference to sulphur) in discussing fertilizers. In practice, however, the use of so-called complete fertilizers regularly involves applying to the soil at least six essential elements, namely, nitrogen, phosphorus, potassium, sulphur, magnesium, and calcium. It is not surprising, therefore, that in ordinary fertilizer usage the need of the three last-named elements does not become apparent. It has been shown, however, that the result is very different, at least in the case of tobacco, when fertilizer salts of high purity are used. On sandy soils subject to severe leaching and containing little organic matter it should not be surprising to find a deficiency of magnesium or sulphur or calcium as well as of nitrogen, phosphorus, or potassium. Sand drown seems to furnish a convincing illustration.

The satisfactory results obtained in inducing sand drown in pot cultures have suggested that by giving due consideration to commercial fertilizer salts with respect to all of the six elements mentioned and by providing for leaching effects pot cultures can be made to yield results more in line with field experience. The essential feature of the leaching process consists in the use of nutrient solutions, complete and with one or more elements omitted, as desired, instead of distilled water for producing leaching effects. The leaching can be pushed as far as desired without

impoverishing the soil except with respect to the elements omitted in the nutrient solution. Obviously the method can not well be applied to compact, clayey soils.

CONCLUSION

Sand drown, a chlorosis of tobacco involving both the green and the yellow pigments of chlorophyll, is due to magnesium deficiency and is markedly aggravated by an increased sulphur supply. For these reasons the use of potash salts of high purity (free from magnesia) and, especially, pure forms of the sulphate have resulted in serious damage to the crop. The need for magnesium is readily met by the use of sulphate of potash containing magnesia or by applying lime containing magnesia. One of the functions of organic matter of vegetable origin, as cottonseed meal, tobacco stems, and barn manure and of lime, is to furnish small quantities of magnesia to the plant through the growing season. The sand-drown disease of tobacco suggests that in interpreting fertilizer action the sulphur, magnesium, and calcium commonly contained in "complete" commercial fertilizers should be taken into account as well as the nitrogen, phosphorus, and potassium. A method of conducting pot cultures in which the soil is leached with complete and incomplete nutrient solutions, as desired, gave good results in the investigation of sand drown and is suggested for use in the study of problems in fertilizer action. It remains to be determined to what extent the findings concerning sand drown of tobacco will apply to other crop plants.

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PLATE 1

Tobacco plant of flue-cured type affected with sand drown. Note that the plant as a whole and the individual leaves have attained normal size and shape and the leaf surface is relatively smooth. The disease begins in the lower leaves and at the tips of the leaves. The veins retain their green color long after the leaf lamina becomes bleached. The leaf lamina usually does not die in local areas or spots as in potash hunger.

PLATE 2

Leaves of tobacco of flue-cured type affected with sand drown, showing successive stages of the disease. The leaf at the bottom has lost practically all green color except along the larger veins and at the base. From bottom to top these leaves fairly represent contemporaneous stages of advancement of the disease on different leaves of the same plant from the base upward.

Sand Drown, a Chlorosis of Tobacco

PLATE 2

Sand Drown, a Chlorosis of Tobacco

PLATE 3

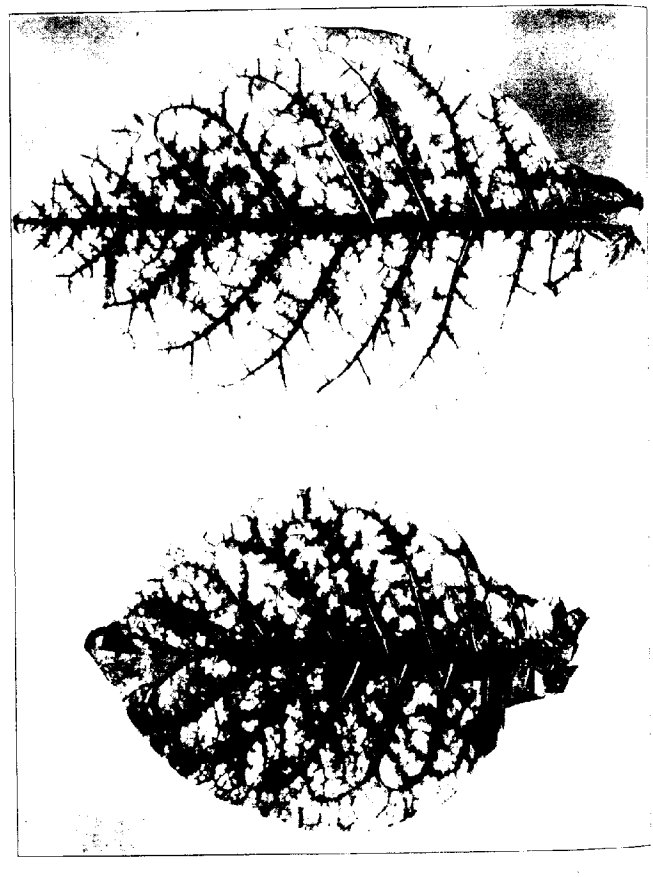

PLATE 3

Leaves of tobacco of cigar-wrapper type severely affected with sand drown, the leaf at the top showing an advanced stage of the disease. Specimen collected at Hatfield, Mass., by Dr. James Johnson.

PLATE 4

Plant at right, which is normal, was watered with the complete nutrient solution described on page 35, including magnesium sulphate, the solution being added in excess so as to produce a leaching effect. Plant at left received the same treatment in all respects, except that magnesium sulphate was omitted from the nutrient solution. Note the advanced stage of sand drown, in which the leaves hang limp. Note, however, that the leaf tissues remain alive and seed formation is successfully accomplished.

PLATE 5

The two plants received the same treatments as the two plants shown in Plate 4, except that the plant nutrients were applied to the soil before setting the plants and the plants were watered with distilled water in limited quantities so as to avoid any leaching action. The plant at left, which received no magnesia, shows pronounced symptoms of sand drown but in less severe form than that of the corresponding plant in Plate 4.

PLATE 6

The two tobacco plants shown were watered with an excess of a nutrient solution containing no magnesia, so as to produce a leaching effect. After well-defined symptoms of sand drown had developed, magnesium sulphate was added to the nutrient solution applied to the plant at right, while the plant at left continued to receive the magnesium-free nutrient solution. At time of photographing plant at right was rapidly recovering. Note the turgidity of the leaves and the partial restoration of the natural color.

PLATE 7

Tip of tobacco leaf, showing chlorosis due to potash deficiency. Note that there are numerous small spots which represent dead tissue. This spotting of the leaves due to localized dying of the tissues is a characteristic feature of potash deficiency symptoms as distinguished from the symptoms of sand drown.

PARASITISM OF SCLEROTIUM ROLFSII ON IRISH POTATOES [1]

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INTRODUCTION

According to various published statements *Sclerotium rolfsii* Sacc. tacks a great number of hosts.[2] It has been frequently reported on e Irish potato, though a clear and full description of the symptoms of effect upon this host has not yet been given. Sometimes the disease s been designated as blight, in other cases as wilt, and very little has en said about the tuber-rot. The type of the decay, as distinguished ım other tuber decays, has not been satisfactorily defined, and the ɔde of the destruction of the host cells has not been followed in detail. view of this scarcity of information the writers feel justified in present- ɜ certain of their observations and experiments relative to this subject.

NATURAL INFECTION

Both the vine and the tuber of the Irish potato may be attacked by is fungus. The disease of stem and foliage may manifest itself in ɾeral different forms which vary with the age of the plant and with e environmental conditions. If very young plants are attacked and e soil moisture is abundant, they are most likely to show damping-off mptoms. Older plants may suffer from a rootrot or a stemrot or ım both with subsequent wilting and drooping of the leaves and the ems, which ultimately lie prostrate on the ground. (Pl. 1, A.)

Natural infection in the field was observed in the South in advanced ages in several States. The symptoms produced were in general those wilt or stemblight. The disease had evidently made its attack at near the surface of the soil, causing a decay of the stem at that point d for a little distance above and below. Frequently the stem was nsumed below to such an extent that little of the underground tissues cept a few strands of vascular fiber remained attached if the tops re lifted. Wefts of mycelium or the sclerotia of the fungus could be en clinging to the stem or extending radially from the plant in and on e surface of the soil.

ARTIFICIAL INOCULATION

To obtain further evidence in regard to the symptoms of the disease rtain field experiments were conducted with artificially inoculated bers. These experiments were carried on in 1919 and 1920 at Arlington

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[2] TAUBENHAUS, J. J. RECENT STUDIES ON SCLEROTIUM ROLFSII SACC. *In* Jour. Agr. Research, v. 18, no. 3, p. 127–138, 1 fig., pl. 3–6, 1919. Literature cited, p. 137–138.

Farm, Va., with two varieties of potatoes, Irish Cobblers and Bliss Triumphs, and two strains of the fungus. One of these latter, culture No. 126,[3] was isolated in July, 1918, from a decaying potato tuber grown in North Carolina and the other, culture No. 127, in May, 1918, from a diseased potato stem grown in Arkansas. The diseased material from both sources represented clear cases of the Sclerotium infection.

Ten tubers of each variety were inoculated in 1919 with Sclerotium No. 126 and 10 with Sclerotium No. 127, making a total of 40 inoculated tubers. In addition, a number of uninoculated tubers of both varieties were planted for control. All these potatoes were grown the preceding season in Aroostook County, Me., where sclerotium-rot has never been found. The inoculations were made a few days before planting, a sclerotium or two being inserted in a narrow channel made in each tuber by means either of a cork borer or a needle. When the potatoes were taken to the field a few millimeters of decay could be noted in every tuber on cutting. The planting was done on May 19, following a period of rainy weather. There was an abundance of moisture in the soil, although the surface of the ground was reasonably flat. The two months following the planting were characterized by prolonged warm and dry weather, intermittent with two rainy periods.

The effect of *Sclerotium rolfsii* on growing potatoes in this test was extremely disastrous. Part of the plantings did not show above the ground at all, part perished from damping-off, and most of the remainder gradually wilted and died before maturity. The progress of the disease during the season was as follows:

June 10. Number of missing hills	11
June 10. Number of small plants (1 to 3 inches) damped-off	7
June 18. Number of larger plants showing wilt	10
June 24. Additional number of wilting plants	6
July 14. Additional number of wilted plants	1
Total number of missing and wilted plants	35

The remaining five plants, although considerably weakened, remained alive until harvest. Their size was only about half that of the plants in the control row, but the foilage and the stems were practically normal in appearance (Pl. 1, B). It is probable that the action of the parasite in these cases was confined to the root system alone.

At digging time, on September 15, no tubers were found in any of the 35 hills wilted or otherwise destroyed by the fungus. The other 5 hills produced a few tubers of the size of a hazel nut, but no rot was in evidence. Control rows produced medium-sized sound tubers, and they showed no signs of damping-off or wilt throughout the growing season.

There was no perceptible difference in the response of the two varieties of potatoes used. However, the severity of the attack of the two fungus strains differed somewhat in this experiment. Thus on June 24 (five weeks after planting), all plants inoculated with Sclerotium No. 126 were either dead or showed distinct symptoms of wilt, while among those inoculated with Sclerotium No. 127, six plants still were apparently unaffected, three of each variety. On July 14 wilt appeared in an additional Bliss Triumph plant; but the other five plants, three Irish Cobblers and two Bliss Triumphs, while under size, showed no other signs of the disease and remained alive as long as the control plants.

[3] Numbers of the cultures in this paper have reference to the writers' collection.

The various symptoms of the disease followed each other in succession. Some seed tubers decayed so rapidly that the sprouts never reached the surface of the ground. Thus, the missing hills, a little over 25 per cent of the total number planted, gave the first indication of the activities of the parasite. About 16 per cent of those that did come up were able to form only a few first leaves, as the stems became thoroughly infested with the fungus, turned dark and water-soaked at and a little below the surface of the ground, and died. The appearance of such plants was fully typical of damping-off. The next distinct stage of attack may quite properly be characterized as wilt. It was observed with older plants, when not only the roots but also the stems were so seriously injured by the parasite that the activity of the conducting vessels was greatly impaired. A little later the stems were so corroded that the plants died. In some cases the foliage symptoms were so typical of wilt that it would be difficult on casual observation to distinguish this form from those caused by Fusarium, Verticillium, or bacteria (Pl. 2, A). It is very easy, however, to ascertain the actual cause of the trouble on closer examination of the base of the stem, where usually a weft of white mycelium and the brown sclerotia of *Sclerotium rolfsii* can be found as well as a stemrot in place of the discoloration of the vascular system. In some cases the infestation of the stem was not deep enough to sever completely the vascular connections; and then the symptoms of the wilt were not so pronounced, but the plants presented a general unhealthy appearance with partly drying, partly wilting foliage. This form may readily come under the conception of blight. This condition, too, led to the ultimate death of the plants (Pl. 2, B). The number of wilted and blighted plants in the foregoing experiment constituted a little over 40 per cent.

A repetition of this experiment was made in 1920 on another piece of land on the same farm. Only the Irish Cobbler variety was used this time, but there were two sets of plantings—an early one, on April 8, and a later one, on May 6. Twelve hills to each strain of the fungus in each series were planted, or 48 hills in all. The remainder of the procedure was identical with that of 1919. The progress of the disease was one of great interest. Up to the middle of June all plants in the earlier set inoculated with culture No. 126 remained healthy, and of those inoculated with culture No. 127 two showed wilt. On the same date in the later set there were 2 hills missing and 7 wilting in the lot inoculated with culture No. 126, while not a single hill inoculated with culture No. 127 showed any sign of infection. During the next two weeks the disease made a general advance in all the sets, and the conditions on June 29 were as follows:

EARLY PLANTING

(a) Inoculations with culture No. 126: 4 hills showed infection.
(b) Inoculations with culture No. 127: 7 hills more or less infected, in some hills only one stem showing the disease.

LATER PLANTING

(c) Inoculations with culture No. 126: 10 hills were affected, of which 5 decayed completely and disappeared.
(d) Inoculations with culture No. 127: No hill showed infection.

At harvesting time, on July 20, 3 hills remained alive in lot (a), an increase of 75 per cent in affected plants, 6 in lot (b) showing that 1 hill, partially affected, had recovered during the last three weeks before the harvest, 4 in lot (c), indicating another partial recovery, and 11 in lot (d),

which signifies death of one plant only. The general succession of the various manifestations of the disease was essentially the same as in the first test and varied with the age of the plants. Thus, there were first some missing hills in those cases where the seed pieces were destroyed promptly in the ground, then damping-off symptoms showed on the young plants, and finally wilt, stemrot, and blight followed in older plants. As to the tuber production and tuber-rot, the following conditions were observed on the date of digging:

EARLY PLANTING

Lot (a): Only 9 hills produced tubers, of small size; a severe rot in 5 hills.
Lot (b): Every hill produced tubers, all undersized; severe rot in 4 hills.

LATER PLANTING

Lot (c): Only 5 hills produced tubers, undersized; severe rot in 1 hill.
Lot (d): Tubers in every hill, varying in size; severe rot in 4 hills.

It appears from the foregoing data that the most serious infection with Sclerotium No. 126 took place in the later planting or later in the season in the earlier planting and, quite the contrary, with Sclerotium No. 127 the most serious infection took place in the earlier planting and very little infection was observed in the later planting. These results indicate, therefore, that the Arkansas strain of the fungus is more adapted to cooler temperatures, while the North Carolina strain is more adapted to warmer temperatures.

The total number of hills which became infected with culture No. 126 in both plantings in this experiment was 19, and the total number of hills which became partially or wholly infected with culture No. 127 in both plantings was only 8. This apparently weaker pathogenicity of the latter strain is quite in accord with the results obtained during the preceding season.

EXISTENCE OF STRAINS OF SCLEROTIUM ROLFSII

The markedly different parasitic activities of the two Sclerotium cultures and their different behavior in the field at different time of the season obviously suggest the existence of strains in *Sclerotium rolfsii*. Taubenhaus [4] summarizing the results of his recent work, stated that—

> There are no varietal nor physiological strains in *Sclerotium rolfsii*.

It is self-evident, however, that this assertion may be regarded as correct with reference only to those strains which were included in his studies and that the generalization of the characteristics of these strains is not at all warranted. As it is seen from the foregoing account of the writers' experiments, their two strains reveal a decided physiological difference. Furthermore, these two strains exhibit a distinct morphological difference, namely, in the size of the sclerotia, as shown in Plate 3, A to C. The individual sclerotia vary in size within the strain, and their average size may be larger or smaller, depending on the kind of medium and environmental conditions; but when considered relatively, on the same medium, at the same age, grown under the same environment, the sclerotia of the culture No. 126 are always larger than the sclerotia of the culture No. 127. Besides, there is a peculiar tendency in the strain No. 126 when food supply is abundant to mass up the sclerotia in large clusters (Pl. 3, C) which has not been noted in the "microsclerotial" strain No. 127. The sclerotia in the clusters are often of a much larger diameter than the individual sclerotia and tend to become elongated.

[4] TAUBENHAUS, J. J. Op. cit.

curved, or irregular in shape. The constant relative difference in the size of the sclerotia of the two strains was observed on the following media: Raw potato, cooked potato plugs, potato agar, corn-meal agar, oatmeal agar, rice agar, and rice. Other media were not included in the studies.

Considering the characters of the two cultures stated in the preceding paragraphs, the writers believe the conclusion to be quite warranted that the difference between Sclerotium No. 126 and Sclerotium No. 127 is that of, at least, varietal character. Having no authentic culture of *Sclerotium rolfsii* Sacc. they are unable to determine which, if either, of the two is the true species.

CHARACTER OF SCLEROTIUM TUBER-ROT

The potato tubers infected with *Sclerotium rolfsii* in the field or artificially inoculated in the laboratory are subject to a rapid progressive decay. This decay is practically odorless and colorless in its earlier stages but takes on a yellowish appearance in older portions, particularly if there is a considerable development of the fungus hyphae. The affected tissues become usually more or less porous. This was exactly the type of rot from which culture No. 126 was isolated. It was also repeatedly reproduced in laboratory inoculations. The term "white rot" would seem to be quite appropriate to and descriptive of this stage of the disintegration. Under sufficiently favorable moisture and temperature conditions the decay may develop into what has been termed "melter" type, when the affected portions become exceedingly soft and watery.

Most of the laboratory inoculation experiments were carried on with the Irish Cobbler variety, but a small quantity of Bliss Triumph and a seedling variety were also tested with no apparent difference in the response of these varieties to the action of the parasite. Inoculum was inserted either in needle pricks or in shallow scalpel wounds. Both relatively young sclerotia and young mycelium of the fungus were used with practically the same success. The inoculated tubers were placed in ordinary glass moist chambers or stone jars with layers of wet filter paper below and above and were left in the laboratory for two weeks at the temperature of 20° to 22° C. The difference in extent of rot produced under these conditions by the two Sclerotium cultures was only slight and may not alone be regarded as significant. The diseased areas resulting from inoculation with culture No. 126 averaged 23 to 25 mm. in diameter and 14 to 15 mm. in depth; those resulting from inoculation with culture No. 127 averaged 18 to 20 mm. in diameter and 10 to 12 mm. in depth. The "melter" type of decay did not develop during this period and under these conditions. Liquid, however, separated from tubers kept in moist chambers for a longer period of time.

The destructive effect of the fungus on potato tuber tissue was very clearly shown when sterile raw potato blocks were inoculated in large quantities in Erlenmeyer flasks. There first developed an abundant growth of pseudo-parenchymatous mycelium enveloping the blocks and practically filling the flasks. Abundant sclerotial formation followed with the development of large compact aggregates of these bodies which were frequently an inch or even more in diameter. When the mycelial development had reached its maximum there appeared a gradually increasing accumulation of light amber liquid in the bottom of the flask, sometimes amounting to as much as 500 cc. in a single 2-liter flask. Since no additional water was placed in the flask with the raw potato blocks, it is evident that this liquid resulted from the action of the fungus

on the potato. Observations made at various stages in the process showed that the mycelium enveloped but did not penetrate the blocks. The middle lamellae first softened, then the cell contents and cellulose walls. The starch grains were corroded and eventually entirely dissolved. If the mycelial mass surrounding the space originally occupied by an individual block was broken open at the proper stage it was found to inclose a cavity approximately the size and shape of the original block but containing nothing but a residue of liquid. Earlier stages showed free starch granules or disintegrating tissue. The starch appears to be the last of the solids to disappear. If the cultures were allowed to remain undisturbed for a sufficient period, at least partial autolysis of the mycelium and sclerotia occurred and the flasks instead of being practically filled contained a more or less compact aggregate of mycelial slime and embedded sclerotia of 200 or 300 cc. volume nearly submerged in a few hundred cubic centimeters of brown liquid.

Examination of the raw potato blocks at various stages of disintegration indicated that the fungus accomplishes its work without penetrating the tissue. The logical inference is that digestive enzyms are secreted which dissolve the host tissue and the digested material becomes available to the fungus by diffusion and osmosis. Taubenhaus [5] made no special study of the enzymic activity of the fungus but states that—

> infection seems to be favored by an enzym secreted by the advancing mycelial strands. . . . Fungous hyphae are not found within the cells but only between them where the middle lamella has disappeared.

Under the artificial conditions of the writers' flask experiments even such mycelial penetration appeared confined to a very shallow surface layer or failed entirely.

Enzym preparations of the hyphae obtained by the acetone-ether method and enzym-bearing powders obtained from the amber liquid by the alcohol precipitation method were used in studying the action of the fungus. Disks of raw potato in water were treated under aseptic conditions with each of the enzym preparations as well as with filtered water extracts of each of them. Such disks invariably softened to the consistency of curd, whereas control disks similarly handled but with the addition of no enzym-carrying preparation or extract remained firm. Similar results were obtained when toluene was used as an antiseptic without special precautions against the entrance of organisms. The disks disintegrated through the softening of the middle lamellae and the consequent freeing of the individual cells, just as may be seen in the initial stages of decomposition in the presence of the fungus itself.

SUMMARY

(1) Infection of the Irish potato plant with *Sclerotium rolfsii* Sacc. may manifest itself in a variety of symptoms which, according to their appearance, may be grouped as seed-piece rot, damping-off, stemrot, wilt, and blight.

(2) Infection of the potato tuber results in a progressive soft white rot, which may take on a so-called "melter" form with profuse extrusion of liquid.

(3) The destruction of the host tissues may be accomplished without hyphal penetration by means of digestive enzyms.

(4) Existence of varietal strains in the fungus known as *Sclerotium rolfsii* is quire apparent from the physiological and morphological differences of the cultures studied by the writers.

[5] TAUBENHAUS, J. J., op. cit., p. 132-133.

PLATE 1

Sclerotium rolfsii on potato:

A.—Wilt and stem decay at the base from artificial inoculation of seed tubers.
B.—A dwarfed plant grown from an inoculated seed tuber.


B
A

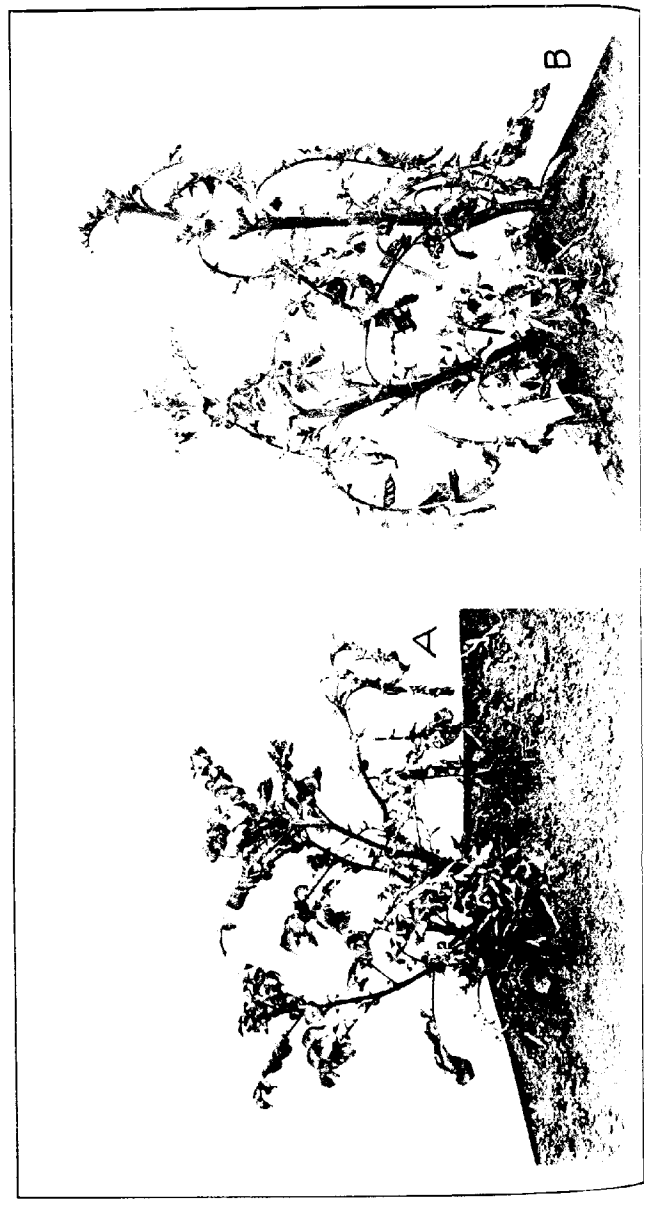
B
A

PLATE 2

Sclerotium rolfsii on potato:

A. Typical symptoms of wilt, result of artificial inoculation.
B. A severe blighting condition, result of artificial inoculation.

PLATE 3

Cultures of *Sclerotium rolfsii* on raw potato slices covered with sterile filter paper.

A.—Culture No. 127 from a diseased potato stem grown in Arkansas.
B, C.—Culture No. 126 from a diseased tuber grown in North Carolina.
C.—Clusters of the sclerotia.


C
B
A

XAMINATION OF AUTHENTIC GRAPE JUICES FOR METHYL ANTHRANILATE[1]

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In a preliminary communication by the present authors[2] attention was rected to the occurrence of methyl anthranilate in grape juice, and it was ien observed that this compound is contained to the largest extent in ices of the Concord type, although the quantity present in a number other varieties was sufficient to permit of detection by the delicate st employed.[3] Through the kind cooperation of Dr. J. S. Caldwell, of ie Bureau of Plant Industry, United States Department of Agriculture, e have been provided with a large number of samples of genuine grape ices which were prepared from definite varieties of the fruit and in ifferent sections of the country. It has thus been made possible for us) obtain further information respecting the distribution of methyl nthranilate in grapes of different botanical origin.

The grape is preeminently a North American plant, for among the mber of species of the genus Vitis found in the world, which is generally nsidered by botanists to range from 40 to 60,[4] more than half are natives this continent. The number of varieties of the grape described in merican viticultural literature is said to be more than 2,000, while twice many more are mentioned in European treatises on the vine.

Inasmuch as the principal object of the present investigation was to etermine whether methyl anthranilate is a common constituent of the ape or whether its occurrence is restricted to certain distinct species the genus Vitis, or the varieties derived therefrom by cultivation, nsideration may first be given to the recognized botanical origin of ie grapes examined, which are designated by their popular names.

A very complete elucidation of the subject of American grapes is con- ined in a work by U. P. Hedrick,[5] to whom we are indebted for much the information concerning the botanical relationships of the numerous arieties of grapes used in the present investigation. Hedrick[6] has also ublished a smaller and more recent work on this subject.

In the classification of grapes a primary distinction is made between ose of the Old World and those of the New World, and it has been noted at the grape is probably influenced to a greater degree by soil, climate, nd culture than any other fruit. In the Old World a single species of

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[1] Accepted for publication Aug. 12, 1922.

[2] POWER, Frederick B., and CHESNUT, Victor K. THE OCCURRENCE OF METHYL ANTHRANILATE IN GRAPE JUICE. *In* Jour. Amer. Chem. Soc., v. 43, p. 1741–1742. 1921.

[3] POWER, Frederick B. THE DETECTION OF METHYL ANTHRANILATE IN FRUIT JUICES. *In* Jour. Amer. hem. Soc., v. 43, p. 377–381. 1921. Bibliographical footnotes.

[4] In Index Kewensis, pt. 4 and sup. 1–5. Oxford. 1895, 1901–15, several hundred species of Vitis are enu- erated, but, as stated by Hedrick,[5] the number depends upon the arbitrary limits set for a species and r knowledge of the genus is as yet too meager to set these limits with certainty.

[5] HEDRICK, U. P. THE GRAPES OF NEW YORK. xv, 564 p., 101 col. pl., 1 port. Albany. 1908. Bibliog- phy and references, p. 531–536. (N. Y. Agr. Exp. Sta. Rpt. 1907, pt. 2.)

[6] HEDRICK, U. P. MANUAL OF AMERICAN GRAPE-GROWING. xiii, 458 p., 54 fig., 32 pl. 1912.

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grape is cultivated—the *Vitis vinifera* L. and its varieties. This species is grown for the production of wine and for making raisins because its varieties have a higher sugar and solid content than those of the American species, which are cultivated chiefly for table use. The European varieties are considered to have a more delicate and richer vinous flavor and a more agreeable aroma, with less acidity, than American table grapes, although the latter are more refreshing and their unfermented juice makes a more pleasant drink.

The most commonly cultivated native American species of grape is the *Vitis labrusca* L., which, in the wild state, occurs in black-, white-, and red-fruited forms. Its numerous varieties possess a characteristic flavor and aroma, often described by the terms "foxy" or "musky," and if not too pronounced this musk-like flavor is often very agreeable. A typical representative of this species is the Concord grape. Another northern species, the varieties of which are esteemed for wine making, is *V. riparia* Michx. The variety under cultivation which is considered one of the best representatives of this species is the Clinton grape.

Many attempts have been made to grow the European grape in the eastern part of North America, but for various reasons these have resulted in failure, the climate particularly not being suitable.[7] It is only in the regions west of the Rocky Mountains, and more particularly in California, that the varieties of *Vitis vinifera* are successfully cultivated in America, and the great viticultural interests of the far West are said to be founded upon the success of this one species, with which the native grape can not compete for any purpose. Nevertheless, American species are regarded as indispensable in this western region for stocks upon which to graft the *vinifera* varieties, and it is said to be probable that the time is not far distant when all California vines will be growing upon roots of American species. The number of *vinifera* varieties grown for wine, raisins, and table grapes extends into hundreds, and it has been noted that the great success met with in the cultivation of this species west of the Great Continental Divide makes all the more remarkable the fact that in no place east of the Divide will varieties of it thrive.

One of the species of Vitis which for many years has been the favorite grape in many sections of the South is *V. rotundifolia* Michx. This is represented by the so-called Scuppernong or Muscadine grape. The fruit of all the varieties of this species is characterized by a strong musky aroma, but it is lacking in both sugar and acid. It is not suitable for table use, chiefly because the berries ripen unevenly and when ripe drop from the clusters. Another southern species is *V. aestivalis* Michx., best represented by the Norton grape.

I. GRAPES OF THE EASTERN STATES

The following varieties of the grape, which are cultivated in the eastern part of North America, were examined for methyl anthranilate. The juices from all these varieties were personally prepared by Dr. J. S. Caldwell from ripe fruit grown at Vineland, N. J., and were therefore perfectly authentic.[8] In this enumeration the accepted botanical derivation of each variety is noted, together with the general characters of the fruit.

[7] Further efforts in this direction have recently been made at the New York Agricultural Experiment Station with more promise of success.

[8] All these juices were cold-pressed and were pasteurized by heating at a temperature of 75 to 85° C. Samples of juice of the Worden and Concord grapes were also prepared by heating the crushed grapes to 85° before pressing. The results of the tests for methyl anthranilate with both cold- and hot-pressed juices were practically identical.

A. VARIETIES WHICH GAVE A STRONG REACTION FOR METHYL ANTHRANILATE

CONCORD (*V. labrusca*).—This is regarded as a pure-bred *labrusca*. The berries are black with a blue bloom. It is the most widely grown of the grapes of this continent, and furnishes 75 per cent or more of the grapes of eastern America. Grape juice is made almost entirely from this fruit, and since the European grape does not make a good unfermented juice the industry is subject to no competition from California or Europe.

EATON (*V. labrusca*).—This is considered to be a pure-bred seedling of the Concord variety. The berries are large and black and are covered with a heavy blue bloom.

WORDEN (*V. labrusca*).—An offspring of the Concord grape. The berries vary from dark purplish black to black and are covered with a heavy blue bloom.

NIAGARA (*labrusca-vinifera* hybrid).—One of the parents of this grape is the Concord. The berries are light green and are covered with a thin gray bloom.

MARTHA (possibly a *labrusca-vinifera* hybrid).—This is a seedling of the Concord, which it resembles greatly but from which it differs in several particulars. The berries are light green with a tinge of yellow and are covered with a thin gray bloom.

IVES (possibly a *labrusca-aestivalis* hybrid).—The berries are jet black and are covered with a moderate amount of blue bloom.

DELAWARE (*labrusca-bourquiniana-vinifera* hybrid).—The berries vary from small to medium, are light red, and are covered with a thin lilac bloom.

MONTEFIORE (*riparia-labrusca* hybrid).—The berries are black and glossy and are covered with an abundant blue bloom.

MISSOURI RIESLING (*riparia-labrusca* hybrid).—The berries are pale or yellowish green, changing to light red or having a tinge of pink when fully ripe, and are covered with a thin gray bloom.

B. VARIETIES WHICH GAVE A SLIGHT BUT DISTINCT REACTION FOR METHYL ANTHRANILATE

POCKLINGTON (*V. labrusca*).—A so-called white grape and seedling of the Concord. The berries are yellowish green and are covered with a thin gray bloom.

LUCILE (*V. labrusca*).—The berries are dark red and are covered with a thin lilac bloom.

VERGENNES (*V. labrusca*).—The berries are either light red or dark red and are covered with a lilac bloom.

BRIGHTON (*labrusca-vinifera* hybrid).—The berries are either light red or dark red and are covered with a dark lilac bloom.

CAMPBELL EARLY (*labrusca-vinifera* hybrid).—The berries are dark purplish black and are covered with a heavy blue bloom.

WOODRUFF RED (possibly a *labrusca-vinifera* hybrid).—This has been supposed to be a cross of the Catawba and Concord grape. The berries are dark brick red and are covered with a thin lilac to faint blue bloom.

LINDLEY (*labrusca-vinifera* hybrid).—The berries are dark brick red and are covered with a lilac or faint blue bloom.

HERBERT (*labrusca vinifera* hybrid).—The berries are a rather dull black and are covered with a thick blue bloom.

DIAMOND (*labrusca-vinifera* hybrid).—The berries are green and are covered with a thin gray bloom.

MERRIMAC (*labrusca-vinifera* hybrid).—The berries are black and glossy and are covered with an abundant blue bloom.

AGAWAM (*labrusca-vinifera* hybrid).—The berries are dark and dull purplish red, somewhat resembling the Catawba, and are covered with a lilac bloom.

WILDER (*labrusca-vinifera* hybrid).—The berries are large, purplish black to black, and are covered with a heavy lilac bloom.

BARRY (*labrusca-vinifera* hybrid).—The berries are large, dark purplish black to black, and are covered with a heavy blue bloom.

PERKINS (*labrusca-vinifera* hybrid).—The berries vary from large to medium, are dull green, changing to pale lilac or light red when fully ripe, and are covered with a rather abundant gray or lilac bloom.

ISABELLA (*labrusca-vinifera* hybrid).—The berries vary from medium to large, are deep black, and are covered with considerable blue bloom.

EARLY VICTOR (possibly a *labrusca-bourquiniana* hybrid).—The berries are dark purplish black and are covered with a heavy blue bloom.

BRILLIANT (*labrusca-vinifera-bourquiniana* hybrid).—The berries are dark red, rather glossy, and are covered with an abundant lilac bloom.

DIANA (possibly a *labrusca-vinifera-aestivalis* hybrid).—This is a seedling of the Catawba to which it bears a strong resemblance. The berries are somewhat irregular in size, rather light red, and are covered with a thin lilac bloom.

EUMELAN (*labrusca-vinifera-aestivalis* hybrid).—The berries are black and glossy and are covered with an abundant lilac bloom.

CLEVENER (possibly a *labrusca-riparia-aestivalis* hybrid).—The berries are black and rather glossy and are covered with a blue bloom.

ROMMEL (*labrusca-riparia-vinifera* hybrid).—The berries are light green with a yellow tinge, glossy, and are covered with a moderate amount of gray bloom.

ELVIRA (*riparia-labrusca* hybrid).—The berries are greenish, with a yellowish tinge, and are covered with a fair amount of gray bloom.

CLINTON (*riparia-labrusca* hybrid).—The berries are dark purplish black to black, glossy, and are covered with a rather thick blue bloom.

IRONCLAD OR DIOGENES (*riparia-labrusca* hybrid).—The berries are small, jet black, glossy, and are covered with blue bloom.

FRANKLIN (*riparia-labrusca* hybrid).—A minor variety, which is said to resemble the Clinton very closely.

CYNTHIANA (*aestivalis-labrusca* hybrid).—The berries are small and black and are covered with a moderate amount of blue bloom.

NORTON (*aestivalis-labrusca* hybrid).—The berries are small and black, somewhat glossy, and are covered with a heavy blue bloom.

GOETHE (*vinifera-labrusca* hybrid).—This resembles to a marked degree the White Malaga grape of European fame. The berries are large, pale red, and are covered with a thin gray or slightly lilac bloom.

DUTCHESS (*vinifera-labrusca* hybrid, with possibly *bourquiniana* and *aestivalis*).—The berries are pale yellowish green and are covered with a thin gray bloom.

KING PHILIP (a minor variety, considered to be a *vinifera-labrusca-riparia* hybrid).—The berries are purple.

C. VARIETIES WHICH GAVE NO REACTION FOR METHYL ANTHRANILATE

CATAWBA (*labrusca-vinifera* hybrid).—The berries are intermediate in size, dull purplish red, and are covered with a moderate amount of lilac bloom.

ULSTER (*labrusca-vinifera* hybrid).—The parents of this grape are said to be the Catawba pollinated by a wild *aestivalis*. The berries are dark dull red and are covered with a thin, light to dark lilac bloom.

SALEM (*labrusca-vinifera* hybrid).—The berries vary from large to medium, are very dark dull red, and are covered with a medium amount of blue bloom.

HERBEMONT (*V. bourquiniana*).—The berries are reddish black or brown, with an abundant blue bloom.

NECTAR (*labrusca-bourquiniana* hybrid, with possibly *vinifera*).—The berries are intermediate in size, dark purplish black, and are covered with a heavy blue bloom.

NOAH (*riparia-labrusca* hybrid).—The berries are small, light green tinged with yellow, and are covered with a thin gray bloom.

CANADA (*riparia-labrusca-vinifera* hybrid).—The berries vary from medium to small, are purplish black to black, glossy, and are covered with a heavy dark blue bloom.

BERCKMANS (*riparia-labrusca-bourquiniana* hybrid).—The berries are intermediate in size, resemble the Delaware in color, and are covered with a lilac bloom.

In connection with the preceding classification, which is based upon the occurrence or absence of methyl anthranilate, it has been deemed of interest to present a survey of the numerous varieties of the grape examined by arranging them according to their botanical derivation. The variations respecting the occurrence of methyl anthranilate in the different botanical groups may thus readily be seen, and the relative amounts and the absence of the particular ester are indicated by the following signs: ++ indicates a strong reaction; + indicates a slight but distinct reaction; − indicates a negative result.

Labrusca.

++ Concord.
++ Worden.
+ Pocklington.
++ Eaton.
+ Lucile.
+ Vergennes.

Labrusca-vinifera.

++ Niagara.
+ Brighton.
+ Woodruff Red.
+ Herbert.
+ Merrimac.
+ Wilder.
+ Perkins.
– Catawba.
++ Martha.
+ Campbell Early.
+ Lindley.
+ Diamond.
+ Agawam.
+ Barry.
+ Isabella.
– Salem.
– Ulster.

Labrusca-bourquiniana.

+ Early Victor.

Labrusca aestivalis.

++ Ives.

Labrusca-vinifera-bourquiniana.

+ Brilliant.

Labrusca-vinifera-aestivalis.

+ Diana.
+ Eumelan.

Labrusca-bourquiniana-vinifera.

++ Delaware.
– Nectar.

Labrusca-riparia-vinifera.

+ Rommel.

Labrusca-riparia-aestivalis.

+ Clevener.

Vinifera-labrusca.

+ Goethe.
+ Dutchess.

Vinifera-labrusca-riparia.

+ King Philip.

Riparia-labrusca.

++ Montefiore.
+ Clinton.
+ Franklin.
++ Missouri Riesling.
+ Elvira.
+ Ironclad (Diogenes).
– Noah.

Riparia-labrusca-vinifera.

– Canada.

Riparia-labrusca-bourquiniana.

– Berckmans.

Aestivalis-labrusca

+ Cynthiana.
+ Norton.

Bourquiniana.

– Herbemont.

II. GRAPES OF THE SOUTHERN STATES

The grapes of the Southern States, as previously noted, are derived chiefly from the species known as *Vitis rotundifolia* or its varieties. Through the courtesy of Dr. J. S. Caldwell we were provided with samples of so-called Muscadine grape juice which had been prepared by Charles Dearing and Guy F. Yerkes, of Willard, N. C. These samples were designated by the following popular names of the particular varieties of grape: (1) Thomas, (2) Scuppernong, (3) Latham, (4) Mish. All the juices were cold-pressed and were clear, pale yellow or brownish yellow liquids. They had a sweetish, honeylike odor, quite unlike that of the northern grape juices. The test for methyl anthranilate, which was conducted with 500 cc. of the respective samples, gave in all cases a perfectly negative result.

III. CALIFORNIA GRAPES

The grapes of California may be considered as representative of the European species, *Vitis vinifera*, of which a very large number of varieties have been produced through cultivation. Two samples of juice from grapes of the 1921 crop were kindly supplied by Prof. W. V. Cruess,

of the University of California, and reported by him to be true to name. These were designated as (1) Muscat and (2) Petite Sirah. The Muscat juice was expressed without heating and was pasteurized in the container at 175° F. The Petite Sirah juice was prepared by heating the crushed grapes to about 160° F., and pasteurizing in the container at 175° F. The Muscat juice was a pale yellow liquid, having a sweetish odor. When distilled for the purpose of the test, the first portion of the distillate was observed to be very fragrant, the odor being somewhat like that of nutmeg. The Petite Sirah juice was a deep purplish red liquid, without much odor but having a pleasant, very sweet taste. No reaction for methyl anthranilate could be obtained with either of these liquids when portions of 500 cc. were subjected to the special test.

We are indebted to Mr. Elmer Snyder, of Fresno, Calif., for several additional samples of California juices, which were supplied by the California Wine Association and designated as follows: (1) Muscat, (2) Burger, (3) Sauvignon vert, (4) Zinfandel, (5) Alicante Bouschet. The first three of these samples were pale yellow or brownish yellow liquids, while the fourth and fifth had a deep purplish red color and a somewhat distinctive odor. The light-colored juices were cold-pressed, while the purplish red ones were obtained by heating the grapes before pressing. In none of these five samples of grape juice could any trace of methyl anthranilate be detected.

COMMERCIAL GRAPE JUICES

In connection with the examination of juices from definite varieties of the grape it was deemed desirable to ascertain the character of some of the commercial products, of which a considerable number were available.

The products from 13 manufacturers of grape juice in the Eastern or Central States, all of which were either definitely declared to have been made from Concord grapes or were deep red liquids having the characters of pure Concord juices, gave evidence of the presence of methyl anthranilate, but not in larger proportions than had been found in perfectly authentic juices. The same positive results were obtained from a red juice produced in the State of Washington. This juice was stated to have been made from Concord and Worden grapes, and it was collected directly from the press by an official inspector from the Bureau of Chemistry, United States Department of Agriculture. On the other hand, one sample of a California product, although labeled "Concord Grape Juice" and containing methyl anthranilate, was a yellowish liquid having a sweetish, honeylike odor, quite unlike that of a pure Concord juice. Its authenticity and purity were therefore extremely doubtful. Very slight reactions for methyl anthranilate were obtained with two red juices, produced by Eastern manufacturers, which were not designated as Concord; and traces of the ester were indicated in two commercial Catawba juices, both of which were clear, pale yellow liquids containing sulphur dioxid.

Only a few commercial juices gave results which indicated the complete absence of methyl anthranilate. These comprised the following products: (1) Two samples designated simply as grape juice, which were clear, pale yellow liquids; (2) one sample specifically designated as Catawba juice, which was a clear, bright yellow liquid; (3) three samples of red California juices, one of which was stated to have been prepared from ripe wine

grapes of the transplanted European varieties, while another was designated as Alicante grape juice, and the third sample was only claimed to be the juice from ripe California grapes; (4) one sample of red juice obtained from the State of Washington and presumably prepared from a variety of the European grape; the expression of this juice was supervised by an inspector from the Bureau of Chemistry, United States Department of Agriculture.

In the course of the preceding investigation a sample of loganberry juice was examined for the presence of methyl anthranilate but, as was anticipated, with a perfectly negative result.

CONCLUSIONS

In considering the results of this investigation it is of interest to observe that those grapes which are regarded by viticulturists as representing pure-bred *Vitis labrusca* contain methyl anthranilate. With few exceptions it was also found in varying amounts in all those varieties which represent hybrids of *V. labrusca*, and especially when this species predominates. On the other hand, this ester could not be detected in juices from California grapes, which are derived from the European *V. vinifera*, nor in juices from southern grapes, derived from *V. rotundifolia*. A negative result was likewise obtained with the one available sample of juice from the species known as *V. bourquiniana* Munson.

Inasmuch as considerable uncertainty appears to exist with regard to the botanical origin of several varieties of the grape it is possible that a determination of the presence or absence of methyl anthranilate may possess some diagnostic value. The extent to which this compound occurs in any particular variety may, however, be influenced in some degree by the soil and climate, as it doubtless would be by varying conditions of ripeness of the fruit.

Although it may be presumed that methyl anthranilate imparts a distinctive odor to those varieties of the grape in which it occurs, it evidently does not completely represent their odorous constituents. As already noted, it was not found in the juice of the Catawba grape, which possesses a characteristic aroma, and it has been observed that the botanical characters of this variety are those of *Vitis vinifera* crossed with *V. labrusca*. A complete chemical examination of the odorous constituents of grapes, which probably differ to some extent in the numerous varieties, still remains to be accomplished.

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